History of HLA:
Ten Recollections

Paul I. Terasaki, ***EDITOR***

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Paul I. Terasaki, EDITOR, ***Los Angeles***

Jean Dausset, ***Paris***

Rose Payne, ***Palo Alto***

J.J. van Rood and A. van Leeuwen, ***Leiden***

D. Bernard Amos, ***Durham***

Walter and Julia Bodmer, ***London***

Roy L. Walford, ***Los Angeles***

Flemming Kissmeyer-Nielsen, ***Aarhus***
and Arne Svejgaard, ***Copenhagen***

J. Richard Batchelor, ***London***

Fritz H. Bach, ***Minneapolis***

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Paul I. Terasaki

PREFACE

History is made by people.... and usually written by other people. The story of what actually took place could be written by participants or by professional historians many years later. Participants may have been too pre-occupied with what was going on in the trenches to have grasped the larger picture. And their experience may have been too immediate and personal to achieve true understanding. Hence the phrase, "we will have to wait to see what the future historians will say".

Our intention in collaborating on writing this book is to give those of us who were in the HLA front lines an opportunity to voice our experiences from a personal perspective. This drama is something future historians can only speculate upon, based on their own experience. They did not live in the environment under which the work was done, and though they may have a retrospective advantage, they cannot possibly know how it really felt.

Regarding the historical time perspective, we have actually waited about as long as we can, for unfortunately, life spans are limited. We have already lost one of our irreplaceable members, Rugerro Ceppellini. As was said,

"Time doesn't pass on.....

Time stays, we pass on".

We sincerely hope you will find reading this volume of ten reminiscences from differing viewpoints to be a unique, worthwhile experience. Even omissions from individual accounts should provide the perceptive reader with enough insight to piece together what actually did happen.

The instructions to the authors of this book were simple: write whatever you like; no constraints on style, length or content. Although I am the nominal editor, it was agreed that I would not edit anyone's manuscript. Each assumed full responsibility for what is printed. In practically all instances, we have been able to return a galley to the authors to ensure that typographical errors did not creep in during the typesetting. And it is true that we have not looked at each other's manuscripts.

Perhaps some explanation regarding the length of my contribution is in order. My first draft was about 30 pages, but when I started to review the actual proceedings from each meeting, I found I had made many errors, due to faulty memory. This then led to abstracting the meetings, and once started, I could not break off. The manuscripts were due on June 30, 1990 (some were early, many were late), and the entire volume was shipped to the printers 6 weeks later on August 10, 1990.

Those of us involved in the HLA adventure who have collaborated and competed with each other over a span of more than 20 years as this field of HLA developed, invite you, the reader to share our successes and disappointments as we sought to gain understanding of the system called HLA.

I would like to thank Jane Schoenberg for her invaluable editorial assistance with my chapter, Su-Hui Lee for her excellent typesetting, Ed Sato, Beverly Looman, Lulu Geer, and Ellen Carnahan for their creative layout of the pictures.

Paul I. Terasaki

The HLA Adventure

Jean Dausset

Address: 9 rue de Villersexel,
Paris 75007,
France

1965

Jean Dausset

1990

THE HLA ADVENTURE

JEAN DAUSSET

A comparatively small international band of scientists, including myself, who were first joined by hundreds and later thousands of additional adherents, have been privileged to live through an adventure which is without precedent in the annals of human biology. This adventure culminated in the characterization of the main histocompatibility system in man, HLA, and in recognition of the fundamental role of this complex in the survival of the human species. This saga has resulted in several achievements: 1) a definition of the products of the HLA supergene, which is of such infinite complexity that it is still undergoing intensive analysis today; 2) recognition of the importance of HLA in organ and bone marrow transplantation; 3) observation of the association of HLA with human susceptibility to disease; 4) discovery of the central role played by HLA molecules in the initiation of immunological responsiveness. As we now approach the 40th year of this adventure, it seems useful to take a walk through memory lane, and to look back at some of the serendipitous events which culminated in the discovery and continuing study of one of the most important determinants of human survival, the HLA system. Knowledge gained in the course of this adventure has literally revolutionized biology and medicine. It has fostered the growth of transplantation medicine, and has provided the basis for the proposition of a genetically based approach to preventive and/or predictive medicine. It has also facilitated our understanding of the mechanisms of recognition of self and non-self, upon which the survival of the species depends.

The first phase in the saga consisted of two major efforts in serology and in experimental skin grafts and led to the definition of Class I HLA antigens (A,B,C) which occur on the surface of almost all human cells. The second major phase was the discovery of the Class II HLA antigens (HLA-D,DR,DQ,DP), which are expressed almost exclusively on the surface of cells implicated in immunological responsiveness. The third key phase was the application of molecular biology techniques to studies of the HLA complex, directly at the level of the HLA genes. Each phase entailed intensive efforts to understand the implications of each new finding, and their applicability to problems in biology and medicine. It is difficult to find words to describe the joy and excitement which greeted each new observation, as investigators saw opening before their very eyes enormously broad new horizons of research and dramatic progress in our understanding of fundamental biological processes.

Before going into the specificities of HLA, one must acknowledge a number of landmarks which were important precursors. As early as 1936, Gorer had defined, serologically the first histocompatibility antigen in the murine species, followed by George Snell's monumental studies of the H-2 system in this species. It would not, however, be altogether accurate to claim that the HLA pioneers were stimulated by the H-2 model to seek a counterpart in man. In actuality, the efforts to study men and mice progressed quite independently of each other for many years. Indeed, the H-2 system was studied mainly by serological testing of murine erythrocytes, and the HLA system was investigated almost exclusively with antisera directed against human leucocytes.

In 1952, mixing on a glass slide a serum sample obtained from a polytransfused patient who was leucopenic with leucocytes isolated from another individual, I observed an unexpected aggregation of enormous leucoagglutinates. Two interpretations were possible: a) this was due to the presence in the serum of an auto-antibody, which might have been the etiology of the patient's leucopenia, or, b) this might have been an alloantibody produced in the recipient by transfused leucocytes. Since the patient's serum did not agglutinate his own leucocytes, the latter interpretation was favored. Subsequent study of many other sera supported the probability that we were indeed dealing with an alloantibody. Since each antiserum sample only agglutinated the leucocytes obtained from a fraction of the unrelated donors selected at random, it seemed that such alloantibodies recognized "leucocyte groups".

The results of the many following leucoagglutination experiments, listed in ranks of 1+ to 4+, depending upon the intensity of the reaction, soon filled large charts which were glued on the walls of our laboratory. The charts seemed so complex as to resist any effort at classification - probably because of the polyspecificity of the antisera. In an attempt to simplify and obtain a greater specificity, I began, in 1958, a program of serial transfusions of an anemic male patient who had never before been exposed to any blood or tissues, using aliquots of blood obtained from one same donor. Several weeks later, a potent leucocyte antibody appeared in the serum of this recipient. This antibody produced strong agglutination reactions against the leucocytes of the specific blood donor, and also against a significant portion of leucocyte samples obtained from other randomly selected individuals in the French population. This antibody was inactive against the patient's own leucocytes, and was baptized "MAC", not because of any particular Scottish irredentist notion, but to honor three individuals who had served so generously as volunteers in our laboratory for these experiments, and whose names began with the initials M, A, and C, respectively. Thus was born the first leucocyte antigen, currently known as HLA-A2. In the original report of these observations, it was suggested that this MAC antigen might be of importance in transplantation.

Two other laboratories adopted the leucoagglutination technique. In Holland, a brilliant young investigator, Jon van Rood, applied the method to a systematic study of many antisera and was the first to apply computer analysis to the results. Van Rood found that his antisera could identify two series of antibodies which reacted, in the Dutch population, in an allelic fashion. Van Rood proposed the name of 4a and 4b for these leucocyte antigens in 1962. On the other side of the Atlantic, Rose Payne also reported, independently of van Rood, that pregnancy frequently resulted in the appearance of such leucocyte antibodies, as I had predicted but not proved.

During this period, separate and independently from these serological studies, Medawar described the immunological nature of skin allograft rejection in rabbits and stimulated John Marquis Converse to investigate this problem in man. Converse charged a young physician, Felix Rapaport, to study the problem in depth.

At this point, I would like to take the opportunity of this book, dedicated to the history of histocompatibility, to express my personal gratitude to Felix Rapaport and give him the credit he deserves for his pioneer work, which often has not been well recognized.

During a decade in New York, he devoted himself to studying the behavior of human skin allografts. He developed, as early as 1956, a precise technique for the transplantation of small circular skin allografts on the forearms of normal volunteers, and devised precise criteria for the diagnosis of skin graft rejection on the basis of stereomicroscopic observations of the surface vessels of the grafts. The "accelerated graft rejection" was produced in recipients previously sensitized by the same donor. The "white graft reaction", was interpreted as an expression of the height of allograft sensitization in man, and even the "recall flare" was described. These observations also led to a definitive demonstration of the cellular mediation of skin allograft rejection in man.

Of more direct relevance to the story, Rapaport and his colleagues provided in 1962 the first formal evidence of the existence of tissue groups in unrelated human subjects. While trying to test the immunological specificity of allograft sensitivity, they transplanted a skin graft from a given donor to a recipient; two weeks after rejection of that graft, they challenged this recipient with a second skin graft from the same donor, and with four or five grafts obtained from other unrelated donors. The skin graft from the donor of the original transplant underwent accelerated rejection. However, to their surprise, one or more of the skin grafts obtained from the other unrelated donors were also rejected in an accelerated manner. This suggested that the donors of these other skin grafts might have had some tissue alloantigens in common with the original donor. Rapaport, et al, then exchanged skin grafts between donors who seemed to share such alloantigens, and compared the survival time with the results of skin grafts exchanged between the donors who did not seem to induce such "cross-reactions" (i.e. their grafts were rejected in first-set fashion in the sensitized recipients). The prolonged skin graft survival observed in grafts exchanged between donors postulated to "share" alloantigens, compared with the other group, provided evidence of greater compatibility (or similar tissue groups) between those donors who had elicited cross-reactions in the sensitized recipients.

In 1963, I met John Marquis Converse at a transplantation symposium organized in Padua. We exchanged notes on the leucocyte group studies and Felix Rapaport's recent findings of shared alloantigens or tissue groups in man. I invited Felix Rapaport to visit me in Paris in 1964, and we decided to join forces, and to perform large-scale skin graft experiments in the population of leucocyte-typed volunteers available in Paris. Thus began a transatlantic collaborative effort and a close professional and personal friendship which continue to this day.

The first International Workshop on Histocompatibility was organized in Durham in 1964 by Bernard Amos. Although there was some cause for optimism, no apparent correlations were seen in the diverging results obtained by various different leucocyte grouping techniques (except, perhaps, for leuco-aggutination), performed by the participating teams on the same samples of donor leucocytes. During one particularly tense scene, Ruggero Ceppellini dramatically tore our data sheets into shreds, because they were not worthy of publication! However, the faith of our tissue typing band was not shaken by these technical difficulties. A second reunion of our now growing international group was scheduled for the following year in Leiden, organized by Jon van Rood. This time, quite similar results were observed with the same leucocyte samples, using the three main techniques in use, i.e., leucoagglutination, complement fixation and Paul Terasaki's recently perfected lymphocytotoxicity test. The serology of HLA emerged in formal fashion at this meeting. One area of uncertainty remained: Were we dealing with a single system, which Ivanyi and I named Hu-I, analogous to the murine H-2 system, or were there two systems – one being the LA1, LA2 (identical with MAC) and LA3 system described by Walter Bodmer and Rose Payne, and the other being the 4a4b system of van Rood.

In order to resolve this issue, the Third International Workshop was held under the leadership of the late, and sadly missed, Ruggero Ceppellini, in Turin (1967). A number of preselected Italian families was typed in double blind fashion by a dozen teams who, in order to insure the accuracy of their results, had brought with them all the necessary laboratory equipment and supplies, as well as their very best technicians. The results showed conclusively that we were dealing with one single major system of leucocyte groups. It was, however, only some time later that it was established that this unique system consists of at least two loci, existing side by side on one chromosome, thus defining a "haplotype". Three years later (1971), the same single system model with two loci was found to also be applicable to the H-2 system in mice.

In parallel with progress in definition of the HLA antigens, growing interest developed on the relationship of HLA to transplantation. My fortuitous meeting with Converse yielded an opportunity to apply Rapaport's skill in skin grafting and his highly reproducible criteria for the determination of graft survival time to seek evidence of the suggested relationship between our serologically defined leucocyte groups (Hu-1 or HLA) and his tissue groups. My administrative office was metamorphosed into an operating room, and the entire area was periodically disinfected and draped. My large desk and a few stretchers became operating tables, with office lamps serving as the light sources. Felix Rapaport began an historic series of several hundred circular (11 mm) skin grafts, placed singly or in series on the forearms of our Paris volunteers. Skin was removed from preselected donors and placed on the appropriate recipients. Sterile dressings were applied, and the grafts were then examined daily in blind fashion, using stereomicroscopic techniques. Photographs taken during these happenings provide some idea of the tremendous enthusiasm and esprit de corps which characterized this endeavor. Most grafting sessions occurred during holidays, when our volunteers were free to come to Hôpital Saint-Louis. Everyone "knew" that we were involved in a unique venture. The entire laboratory was a true ant hill, in full activity. As we had hoped, this effort, which lasted from 1964 through 1977, fully established the status of HLA as the Main Histocompatibility Complex in the human species, and defined the basic laws of transplantation in man.

As one looks back at this incredible adventure, including the semi-clandestine surgical activity in my office, with the help of a Paris panel of volunteers, who represented all walks of life, and jointed in a shared desire to help humanity, one is left with an unforgettable awareness of the true potential greatness of human beings. All of our participants – more than 400 individuals including parents and children – were truly members of the research team; they had all been warned of the potential hazards of each procedure, yet none hesitated to come forth, and none complained about the inconvenience of their many daily trips to Hôpital Saint-Louis for examination of their skin grafts. Thus began a long series of protocols.

Firstly, with the unrelated donors, we systematically studied the role of pre-immunization with blood elements, erythrocytes, platelets and leucocytes (and within the various leucocyte fractions).

Immunization against the red cells demonstrated the primordial importance, as in transfusion, of ABO-compatibility. Pre-immunization with A or B red cells or with soluble A or B antigens always leads to white graft rejection. Amazingly, immunization with platelets does not induce accelerated rejection and this was later explained by the observation that they do not express HLA Class II antigens. As for leucocyte pre-immunization, the MAC antigen (HLA-A2) was shown to act as a potent transplantation antigen. MAC negative recipients sensitized by MAC positive leucocytes (HLA-A2) rejected skin grafts obtained from other MAC positive donors in an accelerated graft manner, but allowed MAC negative skin grafts to survive in normal first-set fashion. Balner and van Rood used the same technique in monkeys and in man and thus also demonstrated the role of 4a and 4b antigens in transplantation.

Secondly, we extensively studied the second graft rejection among members of the same family. Most of the time, the fathers received skin grafts from at least two children bearing one of the two different maternal haplotypes. This technique had two extreme advantages: 1) it provided the certainty that only one haplotype could be incompatible, which greatly simplified the analysis, and 2) it enabled a comparison to be made of the rejection time between two maternal haplotypes in the same recipient, thus possibly establishing a hierarchy between the incompatibilities.

Careful records were kept of the graft results, which were then correlated with the growing array of data developed on the HLA types of the donors and recipients. Subsequently, teams led by Bernard Amos and Ruggero Ceppellini showed that skin grafts exchanged between HLA-identical siblings survived longer than grafts exchanged between HLA haplo-identical siblings; an even shorter survival was accorded to grafts exchanged between HLA-different siblings.

There were also some memorable visits to New York by a number of our French volunteers for leucophoresis and preparation of transplantation antigens from their isolated leucotyes.

These various projects made it possible to identify conclusively the two main immunological barriers to exchange of tissues between related and/or unrelated individuals: the ABO system and the HLA system.

The only truly remarkable and deeply inspiring aspect of this adventure continues to be the enthusiasm and devotion of the large number of volunteers representing a number of different countries, who made such a firm commitment to serve humanity in our laboratories. It is only thanks to their help that the basic laws of human transplantation were elaborated, leading to the development of a totally new therapeutic approach to human disease, i.e. clinical transplantation.

In the meantime, a team of surgeons and nephrologists in Boston, David M. Hume, John P. Merrill, Joseph E. Murray and their associates performed the first series of successful kidney allografts in identical twins, and a Paris team led by Hamburger developed renal transplantation in dizygotic twins. The serology of leucocyte groups was applied early to studies of Hamburger's patients, with the observation that the onset of diuresis after renal transplantation was slowed significantly, or did not occur at all in recipients of leucocyte group-incompatible kidneys. The technique employed in these early days was very crude. We used a battery of antibodies of unknown specificities against the leucocytes of both donors and recipients. If a positive reaction occurred against the leucocytes of the donor but not those of the recipient, it was concluded that the donor possessed at least one antigen absent in the recipient, and was thus probably incompatible. A good correlation was observed! This observation, the first hint in human transplantation of the role of leucocyte antigens, was presented in New York by Jean Hamburger in 1960. At a later date, it became possible to demonstrate that, as is the case for skin grafts, the survival of kidneys obtained from HLA identical sibling donors is by far superior to the results obtained with HLA haploidentical or HLA-different sources.

It is in the category of *unrelated* donor kidney transplants that it has been difficult to convince our clinical colleagues of the long-term beneficial influence of HLA compatibility. The limited number of patients and range of antisera available during the early years were inadequate to attain statistically significant results. Such significance was achieved, however, when it became possible to study a very large number of cases, with more specific reagents. This, in turn, stimulated the organization of organ sharing exchanges, in order to provide patients awaiting transplantation with an opportunity to receive an optimally HLA-compatible organ. Eurotransplant was created in 1968; in 1969, two other groups, Paris-Transplant and Rhone-Méditerranée merged into France-Transplant. UK Transplant and Scandia Transplant followed shortly thereafter, along with a number of North American organizations.

The first bone marrow transplants were also performed during this early phase. The effort, however, was hampered by the fact that even the most compatible bone marrow transplants possible, i.e. grafts obtained from HLA-identical sibling donors, were associated, against all expectations, with a very severe complication. This was the reaction of the transplanted cells against their host, or graft-versus-host reaction (GVH). To this day, the GVH reaction is not fully understood, and often evades all efforts at prevention and/or treatment.

During the same heroic period of transplantation, the basic techniques of heart, liver and pancreas transplantation were elaborated; this provided the basis for today's exponential progress in these areas. In spite of the many obstacles, the success of renal transplantation, and later of liver and heart transplantation, has encouraged physicians to persevere in their efforts to replace diseased vital organs. The advent of new potent immunosuppressive agents was a key element in facilitating this endeavor. It is remarkable, in this context, that the immunological community has persevered and has maintained its ongoing and painstaking analysis of the HLA system, in spite of the continuing skepticism with which this effort is still viewed by some clinicians.

In parallel with the efforts in transplantation, immunologists became concerned at a very early date with the broader biological implications of HLA. In spite of the scarcity of associations between the ABO system and human disease, I proposed that an intensive effort be made to seek possible associations of HLA and disease. This proposal was based upon the known difference in the incidence of certain diseases, depending upon the geographic location of the populations involved. A striking example is the absence of chronic lymphocytic leukemia in China. Lilly, et al, had also just reported the importance of the H-2 system in the development of murine leukemias. The first studies in man, in 1966 and 1967, were concerned with possible relationships of HLA with acute leukemias and Hodgkin's disease, respectively. The results were not encouraging in the first case and only equivocal in the second instance. As in transplantation, however, there was a persistent, intense but more or less haphazard effort to identify associations between certain HLA antigens and disease. The first research on juvenile diabetes was performed in 1978 by Roy Walford. The first hints of such an association were found by Terasaki in 1972 for psoriasis and by Singal in 1973 for diabetes mellitus. These early beginnings culminated in the striking observation of a 90% association between HLA-B27 and ankylosing spondylitis. A large series of other published reports, many somewhat preliminary in nature, then had to be laboriously checked for confirmation. It was clear, however, that this area was a scientific gold mine, well worthy of further exploration.

The enormous number of possible allelic combinations between the products of the two HLA loci A and B, known in 1972, stimulated a proposal to investigate the comparative incidence of the various antigen products in different populations throughout the world. This culminated in the Evian Workshop (1972), of which I was in charge, where the histocompatibility community gathered to compare the results of HLA typing in 56 different geographic populations. The results showed that, because of its extreme polymorphism, the HLA system is a uniquely potent instrument for studies of biological anthropology. Differences observed in HLA antigen frequency in various distinct populations were striking. There was also a remarkable variability in the actual expression of HLA polymorphism – American Indians, for example, had a markedly restricted HLA polymorphism, when compared with less homogenous populations. The 56th population studied were our primate "cousins", the chimpanzees, whose tissue groups turned out to be amazingly similar to ours. In the course of the Evian Workshop, we also began to perceive the probable existence of a third series of Class I HLA antigens, the HLA-C series.

The second major phase in the HLA adventure was the discovery of yet another major HLA locus(i), in close proximity to HLA-A and B. This locus controls the expression of what is known today as the Class II HLA antigens. The first indication of their existence in man was provided by Bernard Amos and Fritz Bach in their observation that mixed lymphocyte culture tests performed between genotypically HLA-A and B identical siblings could, on rare occasions, stimulate a proliferative response. This result suggested that a recombinant event had occurred in these families, between the two known HLA-A and B regions, and one or more of the genes postulated to govern mixed lymphocyte reactivity. This marked the birth of the HLA-D series. Similar "atypical" results were also observed in mice, where Klein, et al, and Shreffler, et al, independently noted that specific antisera could, on occasion, yield positive reactions in some members of a murine population which was apparently H-2 identical. Thus, the Ia antigens were discovered – a new series of H-2 antigens which, amazingly, are only expressed on B lymphocytes. This represented a dramatic departure from the established dogma of the time, that all known HLA and H-2 antigens were expressed on T as well as B lymphocytes. The human equivalent of Ia was identified shortly thereafter. The basic elements of this group were reported at the VIth International Histocompatibility Workshop, held in Aarhus in 1975, under the leadership of Fleming Kissmeyer-Nielsen. By the time of the VIIth International Workshop, organized at Oxford University in 1977 by Walter and Julia Bodmer, the characterization of this series of antigens was almost complete; they were named the HLA-DR (D-related) antigens. There was some concern at first that it would be difficult to incorporate DR typing into routine HLA serological testing. Indeed, it was necessary to isolate the B lymphocytes, and to eliminate from DR typing reagents the anti-HLA Class I antibodies which were so often present in these sera. For this purpose, one had to systematically adsorb all DR typing sera with blood platelets, which only express the Class I HLA antigens. However, no obstacle could deter the enthusiasm of the HLA serologists, and this new dimension of the HLA system was rapidly assimilated. A renewed worldwide effort then culminated in the identification of the most frequent HLA-A, B and DR haplotypes in Caucasians as well as in many other racial populations, culminating in the VIIIth International Histocompatibility Workshop, convoked in 1980, in Los Angeles, by Paul Terasaki. At this time, two teams, Duquesnoy on the East Coast of the U.S. and Park on the West Coast, noted that certain HLA Class II antisera lacking any obvious DR specificity, exhibited a contrasting distribution within populations studied for DR. Thus were born two new series of Class II antigens – DQ on the one hand, and Dw51 and 52 on the other. A third locus was then discovered, on the basis of an elegant technique which demonstrated lymphocyte reactivity in culture between certain cells known to be DR and DQ identical. This finding, which was reported by Shaw, et al, and by Mawas, et al, could be explained best on the basis of a recombinant event between the DQ locus and a new locus, which was named DP.

Once the ensemble of the Class I and II HLA loci was known, it became necessary to provide a more precise definition of the many alleles determined by this extended system. This enormous effort in taxonomy has continued to attract immunologists, along two separate paths - one following classical serology, and the other based upon cellular techniques, designed to define the antigen(s) responsible for stimulation of lymphocyte proliferation in vitro. As our store of knowledge grew, all that had been learned earlier regarding the relationship of HLA Class I antigens to transplantation and/or to disease had to be reviewed in the context of the new information available for the Class II antigens. Far from being disconcerted or discouraged by this progression of events, our HLA family responded to this new challenge with vigor and enthusiasm!

The results of the extensive skin graft studies performed with Felix Rapaport a decade earlier could now be reassessed on the basis of our new knowledge of the HLA Class I and II antigens. Since we knew the exact survival times of each individual skin graft performed in our human volunteers in Paris, it was now just "sufficient" to determine the HLA Class II types of these donors and recipients, in order to develop a more precise analysis of the relative roles of each of these HLA products in transplantation. In addition to the already known importance of HLA Class I antigen compatibility, evidence was now provided regarding the major role of the Class II antigens. It thus became possible to reinforce further a correlation between skin allograft survival time in man and HLA Class I and II compatibility. The new dimensions of HLA were also incorporated immediately into the selection of optimally compatible recipients for renal transplantation. The statistical data soon showed the usefulness of extended HLA matching for the selection of the best (i.e. most compatible) renal allograft recipient.

As for HLA and disease, we unfortunately had only stored cells from the very unusual cases in frozen state. The entire task of collection and typing of new lymphocyte samples from patients suffering from various diseases had to be started again. Promising results were developed very rapidly, and it soon became evident that Class II antigens generally show a far greater relationship to disease than Class I antigens. This seemed particularly clear for the autoimmune states, where (at least in Caucasian populations) there was a statistically significant increase in the incidence of HLA-DR3. The HLA haplotype *A1,B8,DR3,* which is so frequent in Caucasians, and particularly in Northern Europe, was most prominently involved. This has placed in its historical context the original reports of an association of HLA-A1 with juvenile diabetes, subsequently documented for HLA-B8 and finally for HLA-DR3. One intriguing fact, which is only now beginning to be understood, is the extreme susceptibility to juvenile diabetes found by Svejgaard, et al, in individuals who are *both* HLA-DR3 and DR4. However, most other associations of HLA and disease have been relatively weak; in a few extreme cases, the association has, however, been very strong or actually total, as occurs in the case of DR2 and narcolepsy, for example.

These observations stimulated the notion that there may actually be an HLA-determined predisposition to a disease – particularly within families where one or several members have developed that disease. Thus, both preventive and therapeutic conclusions could be drawn from HLA typing.

It is also important to note that, aside from the HLA antigens themselves, there may be diseases due to neighboring linked genes on the same 6th chromosome. Conditions such as defects of complement, iron metabolism and adrenocortical function were also thoroughly studied by the HLA people.

The practical implications of HLA in transplantation and disease have been overshadowed, however, by the discovery of the fundamental role of the Main Histocompatibility Complex (MHC) in immunology. This discovery, by Benacerraf, MacDevitt and Sela demonstrated the central importance of MHC molecules as determinants of immunological responsiveness. Benacerraf noted that guinea pig strains could be divided into responders and non-responders to simple antigens. MacDevitt localized the immune response genes in mice (Ir) with murine MHC, using synthetic antigens provided by Sela. The MHC thus became a crucial focus for cellular as well as humoral immune responsiveness. The next central question was whether the molecules involved were true products of the Class II genes, or of genes existing in close contiguity with the MHC.

The elegant protocol of Zinkernagel and Dougherty appeared to resolve this issue in 1974. These authors demonstrated that, in order to implement as immunological response, the T-responder cells must bear the same MHC molecules as the antigen-presenting cell. This restriction phenomenon was found to be of such exquisite specificity that there seemed to be little further doubt regarding the key role of the MHC in triggering an immune response. It would only be learned later that it is not the identity of the MHC molecules of the two involved cell populations which is of primary importance, rather, it is required that the host T-lymphocytes must first have "learned" in the thymus to recognize as "self" the MHC molecules which characterize all the diploid cells of an individual. This educational process occurs under normal circumstances during T-cell maturation in the thymus. These observations appear to apply equally to Class I or II MHC molecules.

Little by little, the intricate mechanisms underlying immunological responses became unveiled. MHC Class I molecules, expressed fully on nearly all diploid cells, mediate the presentation on the cell surface of foreign antigens, such as viruses which have infected the cell. MHC Class II molecules, limited to immunologically competent cells and macrophages, present foreign antigens after phagocytosis. The basic dichotomy between cellular and humoral responses thus began to lose some of its mystery. However, we remained at this time unsure as to how exactly T-lymphocytes are capable of recognizing MHC molecules on the surface of antigen-presenting cells, or even how the antigens are actually presented.

Many of these issues were clarified during the third phase of the HLA adventure. This era began with the introduction of methods of molecular biology, creating yet another revolution in the field. Immunologists, who had spent years of study on products of genes were now able to approach the genes themselves, and to follow the road from the product to the gene itself – reverse genetics!

Gradually, the genetic map and then the physical structure of the HLA complex became better understood. The application of molecular biology techniques to this problem was rudimentary and somewhat controversial by the time of the VIIIth International Histocompatibility Workshop, held in Munich and Vienna under the leadership of Albert and Mayr (1984). This workshop was unique, however, in that it provided a common forum, where a broad spectrum of specialists, including serologists, cellular immunologists, biochemists and molecular biologists could exchange ideas and concepts, and, were able, for the first time, to arrive at a true synthesis of their individual observations. As a result of the boost given to classical serology by the new monoclonal antibodies, as well as the now-established lymphocyte proliferative and cytotoxicity techniques, with further help from chemical and electrophoretic techniques of separation of the various molecules, and the new methods of DNA hybridization and sequencing, four contrasting views of the same situation coalesced in Vienna, with the emergence of a new global synthetic concept of HLA.

From the fundamental standpoint, the genes and pseudogenes of the four major gene families within the HLA complex (Class I and Class II DR, DQ, DP) have been localized vis-a-vis each other on the 6th chromosome, and many of their individual alleles have been identified – some have actually been sequenced. At the practical level, the techniques of DNA splitting by restriction enzymes have provided the hope of isolating specific fragments of a given antigen. The first fragment, specific for HLA-B8, was described in our laboratory by Howard Cann and Daniel Cohen. Equally encouraging are the possibilities opened by the specific oligonucleotides unveiled by sequencing of a given allele. It seems clear that the future lies with a continued application of the new techniques of molecular biology to HLA and the immune response. There is little doubt that the era of laborious serological characterization of HLA alleles, based upon a search for immune sera, reacting in more or less similar fashion upon a leucocyte panel is now obsolete. New alleles can, from now on, only be accepted once they have been identified by specific sequencing.

The process of synthesis of the originally serological, then cellular and clinical, and finally molecular techniques for study of the HLA complex, was continued at the Xth International Histocompatibility Workshop, organized by Dupont in New York City (1987). Two sensational events occurred at this meeting. The first was the laborious but now definitive characterization of the T-lymphocyte receptor, which has many structural analogies with MHC molecules, but also shows many differences. Like the MHC molecule, the receptor consists of two polypeptide chains, united by disulfide bonds. As a consequence of its tertiary configuration, it gives specific recognition of the "self" MHC molecule, but ignores cells bearing a different MHC molecule, even if they present the same peptide antigen –i.e. an MHC-restricted antigen presentation is required to trigger an immunological response. The second major event was the impressive culmination of the long-term efforts of Strominger and his team, to provide a crystallographic image of the HLA-A2 molecule. One could now clearly see the tertiary structure of the antigen, and the presence of a foreign peptide within a cavity formed at the distal extremity of the molecule by the helical alpha and beta sheet chains – a veritable nest is formed, wherein one or more peptides produced by intracytoplasmic enzymatic splitting of a phagocytosed antigen are inserted. It is not difficult to imagine the happiness of all participants in the HLA adventure, when faced with this brilliant demonstration of the central role of the MHC molecule in the immune response.

Looking back at this saga, which continues to unfold at present, the HLA adventurers may be justified in feeling a certain sense of achievement. Organ transplantation, including kidney, heart, lung and pancreas, as well as bone marrow, is a major clinical reality today, much aided by the advent of potent new immunosuppressive agents, such as cyclosporine and yet other promising products, leading to ever more potent and targeted immunosuppressive drug therapy. The usefulness of HLA compatibility has been established firmly in renal transplantation. The limited preservation time provided by cold storage for hearts and livers has not as yet permitted the use of HLA typing for the prospective selection of optimally compatible recipients of these organs. This is only a temporary situation, and further progress in preservation and more rapid techniques of HLA typing emerging from molecular biology, should help to resolve this problem in the not-too-distant future. It should be noted that retrospective studies have already demonstrated the value of HLA compatibility in this regard. From the very first months after cardiac transplantation, for example, the survival curves have shown significant differences in the survival of compatible versus less compatible heart recipients.

This success should not, however, permit us to forget that the precise mechanism(s) responsible for the association between HLA and disease remains almost as obscure today, 23 years after publication of the first report, as it was at that time. Even the most pronounced associations, as seen in ankylosing spondylitis or with narcolepsy, still lack a definitive molecular basis. A most troubling and frustrating example, however, has been the relationship between HLA and juvenile diabetes, one of humanity's greatest scourges. By now, thousands of juvenile diabetics and their families have been studied by hundreds of teams under a variety of latitudes and ecological conditions. A number of extremely precise facts have come to light. For example, we know that the absence of asparaginase in position 57 on the DQ beta chain greatly favors appearance of the disease, but this observation does not suffice to explain all the facts. Possibly, as shown by Hors, et al, another mutation, such as the occurrence of arginine in position 52 on the DQ alpha chain, may be required for the configuration of a DQ molecule which is specific for the host's susceptibility to diabetes. As occurs so frequently in biology, there is no direct relationship between what is already known and the practical applicability of this new knowledge. There is no question, however, that steady progress in our understanding of the HLA region of the human genome may lead, within the near future, to new preventive and/or therapeutic techniques.

This notion forms the basis for the predictive approach to medicine which I attempt to publicize. But at this time, predictive medicine depends principally, if not totally, upon HLA markers. Already today, we can detect at birth or in utero, by HLA testing, the chances of a conceptus to develop juvenile diabetes. This approach may be of particular significance in a family where a prior pregnancy has already resulted in the birth of a child with juvenile diabetes. One could visualize a similar effort in the general population – at first within families at greatest risk, e.g. those instances where one parent is HLA-DR3 and the other is HLA-DR4; eventually, global population studies could be feasible.

Predictive medicine has far greater ambitions. It is now necessary to add to the HLA markers the thousands of other marker systems which are distributed so widely along the 3.5 billion base pairs of the human genome. It is in this spirit that I founded the Human Polymorphism Study Center. This center, which draws its strength from the same kind of international collaboration which culminated in the Histocompatibility Workshops, is currently working to develop a map of the human genome, on which it may be possible to localize the genes of monogenic diseases first, and then the genes for polygenic diseases. Progress in this endeavor holds broad implications. It may permit the intrauterine diagnosis of a number of monogenic (i.e. single gene-determined) diseases. In the past, such diagnoses have been based upon indirect detection of the pathogenic gene, by its association with a known neighboring marker. Indeed, this approach also extends to the determination of susceptibility on the basis of an association(s) with an HLA marker(s), where this marker may only be one of the polygenic determinants involved in the disease. There is also a multitude of polygenic diseases which are not associated with HLA, and which may occur in those individuals whose genome bears the set of genes responsible for expression of the disease. Familial hypertension is probably a good example of such a disease. In the rat, four genes have been identified as the determinants of susceptibility to hypertension. The identification of similar genes in man could permit a preventive campaign to eradicate this terrible human disease. Similar factors are likely to be operative in the various forms and expressions of malignant disease. Predictive medicine, as applied to the individual, may thus permit, in practice, an elective form of prevention as well as the early therapy of so many diseases which have been most harmful to humanity. May one even dare dream of identifying those components of the human genome which control aging ...

The HLA adventure is now over 30 years of age; it has already triggered three scientific revolutions, two of which enjoyed almost immediate practical applications. One of these has been the fulfillment of an age-old human dream – organ transplantation. The other has been an improved approach to the predictive aspects of individual human destiny. The third, and probably far more important development, is our growing understanding of the molecular mechanisms of individuality, of recognition of self and non-self, and consequently of the survival of the species.

What a unique privilege for me, in friendly association with the other pioneers, to have lived through the exalting and rebounding episodes of this great adventure!

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CURRICULUM VITAE

Jean Baptiste Gabriel Joachim Dausset

PERSONAL

Born:	October 19, 1916 (Toulouse, France)
Home Address:	9 rue de Villersexel, Paris 75007, France

EDUCATION

1932	Lycée Michelet, Baccalaureat
1945	University of Paris Medical School, M.D.
1948	Harvard Medical School
	Internship and Residency:
1946-50	Internal Medicine and Hematology

POSITIONS

1950-63	Director of Laboratories, National Blood Transfusion Centre
1963-78	Biologiste des Hopitaux, Paris Municipal Hospitals
1963-68	Co-Chairman, Institute for Research into Diseases of the Blood
1968-84	Director of Research Unit on Immunogenetics of Human Transplantation, Institut National de la Santé et de la Recherche Médicale
1969-89	Founder and President, France-Transplant
1982-	Founder and President, Bone Marrow Grafts-France-Transplant
1984-	President, Human Polymorphism Study Center

TEACHING APPOINTMENTS

1958-68	Associate Professor, University of Paris, Faculty of Medicine
1968-77	Professor of Immunohematology, University of Paris, Faculty of Medicine
1977-87	Professor of Experimental Medicine, Collège de France

HONORARY TEACHING APPOINTMENTS

1970-71	Visiting Professor, New York University
1977	Professor Honoris Causa, University of Brussels
1977	Professor Honoris Causa, University of Geneva
1980	Professor Honoris Causa, University of Liège
1982	Professor Honoris Causa, University of Lisbon
1985	Professor Honoris Causa, University of Rome
1986	Professor Honoris Causa, University of Zagreb
1990	Professor Honoris Causa, University of Lima

MEMBERSHIP OF ACADEMIES

1969-	Honorary foreign member of the Belgian Royal Academy of Medicine
1977-	Académie des Sciences de l'Institut de France

1977-	Académie de Médecine (Paris)
1979-	American Academy of Arts and Sciences (Boston)
1979-	Yugoslavian Academy of Arts and Sciences, elected (Zagreb)
1981-	Hungarian Academy of Sciences, elected (Budapest)
1981-	American National Academy of Sciences

SOCIETIES

1978-81	President, French Society of Immunology
1971-82	President, French Society of Haematology

INTERNATIONAL MEETINGS

1965	Co-Chairman, International Tissue Transplantation Conference of the New York Academy of Sciences
1967	Co-Chairman, First Congress of the Transplantation Society
1972	Secretary, Fifth Workshop and Conference on Histocompatibility
1975	Chairman, Sixth Workshop and Conference on Histocompatibility
1976	President, First International Symposium on HLA and Disease
1980	President, Fourth International Congress of Immunology

AWARDS AND PRIZES

1967	Grand Prix des Sciences Chimiques et Naturelles (Académie des Sciences)
1967	Medaille d'Argent du Centre National de la Recherche Scientifique
1968	Grand Prix Scientifique de la Ville de Paris
1969	Prix Cognac-Jay (Académie des Sciences)
1970	Stratton Lecture Award
1970	Landsteiner Award AABB, San Francisco
1977	Gairdner Foundation Prize
1978	Koch Foundation Prize
1978	Wolf Foundation Prize
1980	Nobel Prize for Physiology and Medicine
1987	Honda Prize
1990	Jimenez Diaz Foundation Prize

DECORATIONS

1981	Commandeur, Légion d'Honneur
1977, 1987	Commandeur, Grand Croix, Ordre National du Mérite
1981	Commandeur, Palmes Académiques
1944	Officier, Médaille Ancien Combattant
1972	Dutch Red Cross
1977	Commandeur de l'Ordre de la Couronne, Belgique

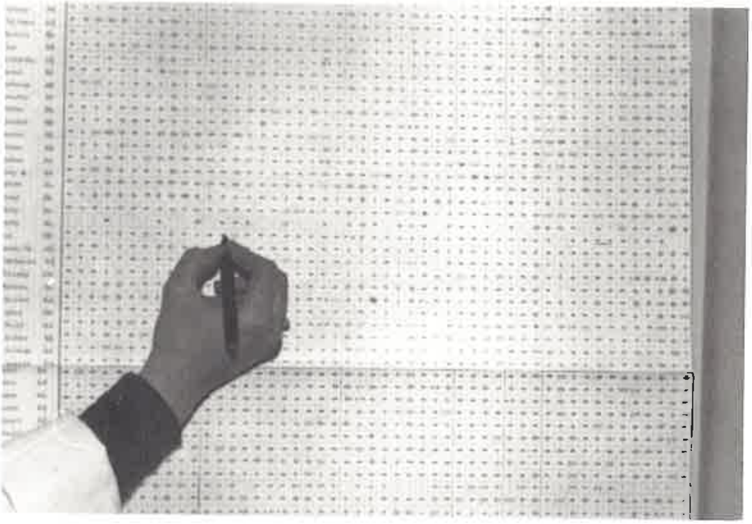
MILITARY CAREER

Captain, Medical Corps, French Army Reserve

Second World War:	French Campaign	1940-41
	Tunisian Campaign	1944
	Normandy Campaign	1945

MEDICAL LITERATURE

1956	Immunohaematology, Biological and Clinical Aspects, Vol I, 719 pages, Flammarion, Paris
1968	Human Transplantation, edited in collaboration with F.T. Rappaport, 1 volume. 728 pages, Grune and Stratton, New York
1972	Histocompatibility Testing 1972, edited in collaboration with J. Colombani, 1 volume. Munksgaard, Copenhagen
1976	Histocompatibility 1976, in collaboration with G. Snell and S. Nathenson, 1 volume. Academic Press, New York
1977	HLA and Diseases, edited in collaboration with A. Svejgaard, 1 volume. Munksgaard, Copenhagen
1980	Immunology 1980, Progress in Immunology IV, edited in collaboration with M. Fougereau. 1 volume. Academic Press, London
1981	HLA 1982, edited by J. Dausset, 1 volume. Flammarion, Paris
1984	Leucocyte Typing, edited in collaboration with A. and L. Bernard, C. Milstein and S.F. Schlossman. Springer-Verlag, New York, Tokyo
1985, 1990	HLA, edited by J. Dausset and M. Pla, 1 volume. Flammarion, Paris, 1985 and 1990



Dausset's
own
computer
Hopital
Saint-Louis
1965

Jean Dausset
Felix Rapaport
performing
leukophoresis

Jean Dausset
Collecting
samples on
Easter
Island
for Fifth
Workshop
1971

Felix
Rapaport
performing
a skin
graft

Early History of HLA

Rose Payne

Address: Stanford Medical School Blood Center
800 Welch Road
Palo Alto, California 94304

Rose Payne

J.J. van Rood **Rose Payne**

EARLY HISTORY OF HLA

ROSE PAYNE

In the early 1950s, I was carrying out research on the autoantibody involved in acquired hemolytic anemia. This was in the laboratory of Dr. R. Evans at Stanford. He had proposed that autoantibodies might also be found in leukopenic and thrombocytopenic patients. We had pursued studies in idiopathic thrombocytopenic purpura but had not tackled the problem of leukopenia. Then, examining the European literature, I noted the description of a technique for the demonstration of antibodies to white blood cells and the claim by Professor Dausset that these were related to leukopenia. To me these were intriguing bits of information. I set up his test for a few sera in our collection. To my surprise the leukoagglutinins he described were fairly common. Enthusiastically I wrote to Dausset to confirm the finding of such antibodies. But there were doubts in my mind as to a causal relationship to leukopenia for many of our patients were not leukopenic. In 1955 these findings led me to organize a systematic study to examine the sera from hematologic patients with special emphasis on leukopenia. I suspect I became a nuisance among the hematologists who brought their patients to Stanford for I was continuously inquiring if they had any leukopenic patients. I managed to collect 350 patients with various hematologic disorders and added to these the sera from 50 healthy medical students for controls. This study was published in 1957 in the *Archives of Internal Medicine.* Leukocyte antibodies were found in over 10%, i.e. 38 of the 350 patients. These were absent in the 50 healthy controls. The patients with antibody were studied for age incidence, sex differences and blood group with no significant correlation with any of these factors. The anemias, the leukemias, the idiopathic thrombocytopenic purpuras and the collagen diseases accounted for most of the patients with leukoagglutinins. At the time of testing, of the 36 for whom leukocyte counts were available 23 or 64% were neither leuco- or neutropenic. Examination of the overall history of these patients showed that 16 or 44% were never leukopenic or neutropenic. These antibodies did not agglutinate the cells of the serum donor and therefore did not appear to be autoantibodies. The prior occurrence of multiple transfusions in these patients suggested that the antibody was possibly the consequence of isoimmunization.

What was particularly interesting was the occurrence of febrile transfusion reactions among many of the patients. Febrile transfusion reactions were the most common complication of blood transfusions. At that time, experts on these unexplained reactions attributed them to either bacterial pyrogens, or to red blood cells or to plasma factors. To establish that the transfusion reactions were instead due to administration of white blood cells, I asked for permission to verify this in patients by dividing a required unit of blood into a leukocyte poor and a leukocyte rich fraction. If the patients suffered a febrile transfusion reaction to the leukocyte rich fraction only, we should have proven our point. Permission was not granted. The conservatism of the Chief of Medicine was frustrating.

Shortly after this refusal I received a telegram from Professor Dausset. In 1956 he was in America attending meetings and announced he would be delighted to visit me. When he came, I described my findings and my interpretation that isoimmunization by transfused white blood cells was responsible for the leukoagglutinins. I told him of my dilemma and asked if perhaps he could test my hypothesis. He returned to France and carried out the experiment which I was not allowed to undertake. My prediction proved true. When the fraction of the blood unit with a large number of white blood cells was transfused to a patient with antibody, the patient suffered a reaction of chills and fever. The leukocyte poor fraction yielded no such response. This having been demonstrated, I was allowed to test our patients.

To confirm and extend the view that leukoagglutinins might be a significant factor in the etiology of the unexplained febrile transfusion reaction, we continued our studies on the association of febrile transfusion reactions and leukoagglutinins which was also published in 1957.

We pursued three lines of investigation. First, a group of sera from patients with a febrile transfusion reaction was examined for leukoagglutinins. The outstanding feature of the data was the surprisingly large number of sera in which leukoagglutinins were found. Among 49 patients who received repeated transfusions, 35 or 65% had developed leukoagglutinins. In contrast, red cell antibodies were demonstrable in only seven patients. Five of these latter patients also had leukoagglutinins. Red cell antibodies alone were observed in only two instances.

Secondly, patients receiving repeated transfusions were followed for the development of both leukoagglutinins and febrile transfusion reactions. The first appearance of leukoagglutinins often occurred at about the same time as the first transfusion reaction. Leukoagglutinins usually developed after eight or more transfusions. Of 15 patients studied only two failed to have reactions.

Lastly, attempts were undertaken to prevent these reactions by the administration of blood from which the majority of leukocytes had been removed. The first patient treated in this manner had suffered more than one severe febrile transfusion reaction preceded by shaking chills. He possessed a leukoagglutinin which reacted in vitro with all test leukocytes. He had also developed a febrile transfusion reaction to a unit of washed red cells containing the usual quota of leukocytes. This procedure, commonly in use in those years, also caused a febrile reaction.

Subsequently this patient was treated with leukocyte poor units of blood on 11 occasions during the succeeding four months without provoking any febrile response. The leukoagglutinin was constantly present throughout this period. Based on this success, 11 patients with leukoagglutinins and febrile transfusion reactions were treated similarly; febrile transfusion reactions failed to develop in any of them. When it became necessary to administer whole blood rather than leukocyte-poor blood to 2 of the 11 patients, febrile transfusion reactions did not develop. One explanation for this failure was the possible occurrence of leukocyte types.

To define types, sera containing leukoagglutinins were tested against a large panel of leukocytes. To our amazement the sera tested agglutinated more that 80% of the cell donors. Given this situation one could not define leukocyte antigens using sera from multitransfused patients.

We continued to study febrile transfusion reactions with the view of comparing the frequency among men, women and children. Sera were collected from 221 transfused patients. There appeared to be a greater frequency of women in this series. Leukoagglutinins were present in 90 out of 125 women, 47 out of 82 men and 10 out of 14 children. This is shown in Table 1.

All transfusions administered within a 5-day period were considered as a single stimulus. Using this definition, a patient with a single transfusion stimulus may actually have received one or several units of blood. The key observation here was that

Table 1. Distribution of leukoagglutinins among men, women and children with febrile transfusion reactions

Patients	Total No. of Patients	Patients with Leukoagglutinins	Proportions
Adult women	125	90	90/125 = 72%
Adult men	82	47	47/82 = 57%
Children	14	10	10/14 = 70%
Total	221	147	

about half or 44 of the 90 women with leukoagglutinins had a reaction to their first or second transfusion stimulus. This was in marked contrast to the 47 men with leukoagglutinins, none of whom showed evidence of reaction to so few transfusion stimuli. In this group of men there were 64 who had received between nine and nineteen transfusions; 39 who received more than 20 transfusions. Of these 39, 21 had more than 50 transfusions. At all these increased levels, appreciable differences between the men, women and children did not appear.

In searching for an explanation as to why women had leukoagglutinin transfusion reactions after very few transfusion stimuli, it seemed plausible to consider that leukoagglutinins might have developed during pregnancy. This led to a review of the obstetrical history of the women. Of the 44 women who reacted to their first or second transfusion stimulus, 38 had had one or more pregnancies. Among the remaining women, there were no known prior pregnancies. These findings were consistent with the idea that stimulation by fetal leukocytes may have been responsible for the reactions in women with so few transfusions. However, the coincidence of past pregnancy, leukoagglutinins and febrile transfusion reactions after few transfusions did not establish a cause and effect relationship. More decisive evidence to support the hypothesis would be the demonstration of agglutination by the maternal serums of the leukocytes of the husband and/or the child. This was illustrated in nine families. In eight of the nine, the maternal serum agglutinated the leukocytes of one or more members of the family.

A further 40 patients with febrile transfusion reactions were treated with leukocyte poor blood. A leukocyte count of 750 per cubic millimeter in the blood to be transfused was sufficiently low to prevent reactions in all the patients tested. From this report in 1960, it became clear, that in the absence of red cell antibodies, febrile transfusion reactions were due to an antibody against white blood cells. Women had a leukoagglutinin febrile transfusion reaction in greater proportion than men or children. These could be related to prior pregnancies.

It seemed to us that if a child was the stimulus for leukoagglutinin formation, sera from gravid women would have a more limited reaction frequency than sera from a multitransfused individual. Perhaps these would lend themselves to definition of leukocyte types. For this we began a study of the development and persistence of leukoagglutinins in parous women. We haunted the obstetrical clinic, collecting sera from all the gravid women and spouses who were willing to be tested. There we had a number of experiences. Many men were afraid to be tested. We became inquisitors, asking if the mother had more than one husband, if she had had a series of transfusions and other personal bits of information. We proceeded with delicacy and

Table 2. Relationship of parity to occurrence of leukoagglutinins in 456 women

No. of the Current Pregnancy	No. of Patients Investigated	Patients with Leukoagglutinins	Percentage of Patients Immunized
0	50	0	0
1	86*	1	1
2	68	13	19
3	37	8	20
4	38	9	24
5 or more	177	43	24

* Forty-nine of these were examined for the first time after delivery.
The single case with leukoagglutinins was demonstrated postpartum.

caution. We learned of children born out of wedlock and other odd bits of data.

What became clear from this work published in 1962 was that usually leukoagglutinins appeared in gravid women after a second pregnancy. The relationship of parity to the occurrences of leukoagglutinins in 456 women is shown in Table 2.

Of 68 women tested during the second pregnancy, 19 had leukoagglutinins; no antibody was found in nongravid women. There was not a significant increase in the percentage of women with leukoagglutinins during succeeding pregnancies. The problem posed by finding that two pregnancies were usually required for the production of leukoagglutinins was, did the second child alone or did both children possess the potentially stimulating leukocyte antigen. To answer this question, family studies were undertaken to test if the leukocytes of the husband and the offspring would be agglutinated by the maternal serum. The antigen was present in the husband and in both first and a succeeding child. Invariably when a leukoagglutinin was found at least two children, not necessarily the first two, possessed the provoking leukocyte antigen.

An inquiry into the earliest time during the second pregnancy when leukoagglutinin might be detected was not specifically undertaken inasmuch as most women were tested during the last two months of pregnancy. However some data on the occurrence of the antibody early in the second pregnancy were accumulated. In one subject the leukoagglutinins were observed in the third month of gestation, in another during the fourth month and in two others during the fifth month. Their presence early in gestation suggested either the passage of cells or antigens during the course of fetal development followed by an anamnestic response to them and/or continuing manufacture of antibody in response to the sensitizing dose received at parturition of the first child.

The leukoagglutinins of many women following delivery showed considerable change in their capacity to react with a panel of cells. Partial loss of activity of the leukoagglutinin, – that is failure to react with as many members of the panel as previously – often preceded complete disappearance from the serum. Leukoagglutinins persisted in the sera of parous women for varying periods of time after parturition. The antibodies of 16 of 31 women could still be identified in their sera three years after delivery. The distribution of potentially stimulating leukocyte antigens in the offspring of two large families provided suggestive evidence that the formation of leukoagglutinin had persisted in two women for eight years after sensitization. The leukoagglutinins found in gravid women had numerous specificities. Forty agglutination patterns of leukoagglutinins were observed in the sera of 68 women; 18 were duplicated using a panel of 10 leukocyte donors.

Having demonstrated that leukoagglutinins occurred in fairly large numbers in gravid women with two or more pregnancies, i.e. about 15%, we became curious as to whether the antibodies might have adverse clinical consequences in the newborn, in a manner analogous to Rh immunization. During 1959 and 1960, five cases dealing with leukopenia and/or neutropenia accompanied by infection in newborn infants from mothers with leukoagglutinins were published. Some workers, including vanRood, Jensen, Rossi and Brandt were at variance with this view. It seemed pertinent on consideration of the conflicting information available to assess the frequency with which transplacental passages of leukoagglutinins might be demonstrated and the frequency with which an effect upon the newborn might be discerned. Data on 150 offspring from 147 parental pairs were examined. When leukoagglutinins were found in the serum of a gravid woman, her infant was tested for the agglutinin provoking leukocyte antigen. Newborn infants with antibody provoking leukocyte antigen constituted the test group and infants from women without demonstrable white cell antibody made up the control group. Total leukocyte counts and the differential counts of newborn infants who provided the stimulus for their mothers to produce leukoagglutinins were compared with the counts of infants who failed to stimulate such production. The leukocyte counts of 39 full weight newborn infants who stimulated their mothers to form leukoagglutinins were compared with the counts of 76 newborns who failed to do so. A significant difference between the mean total leukocyte or differential counts for the two groups was not observed. Transplacental passage of the leukoagglutinin was demonstrated in 15 of 49 newborns examined. It was concluded that in this series, transplacental passage of leukoagglutinin from sensitized mothers to newborns with antigen did not induce significant leukopenia or neutropenia. A leukoagglutinin-related disease of the newborn was not identified.

By 1963, it had become clear that transfusions and pregnancies induced leukoagglutinin formation. When an individual received a blood transfusion following leukoagglutinin formation, a febrile transfusion reaction frequently occurred. Gravid women with leukoagglutinins but no prior transfusions might also have a febrile transfusion reaction if the appropriate white cell type had been given. These reactions could be avoided if leukocyte poor blood was infused.

The next study was an attempt to define leukocyte types. We had been struggling with this problem for some time. In 1962, van Rood had published a thesis describing a series of white cell antigens using a computer analysis of his data. In this publication he seemed to define two allele systems which were called Group 4 through Group 9. I enlisted the assistance of Dr. Bodmer to set up a computer analysis of our data. The reactions of 35 antisera with from 150 to 200 leukocyte donors were summarized in the form of 2 x 2 tables. Individual reactions were recorded and a chi square for each association was calculated as to whether a positive or negative value was present. There were no sera which were unequivocally identical. There were some pairs where the reactivity pattern of one serum was contained within another. Nineteen of the sera were resolved into three groups which were such that all or nearly all of the sera within a group showed a positive pairwise association. The 2 x 2 chi squares for these sera are shown in Tables 3 and 4.

These three groups defined three different antibodies and hence three corresponding antigens. Two were called LA1 and LA2. The third was van Rood's 4b.

Table 3. X^2 Relationships of LA1 and LA2 sera

Serum Number														
LA1	2													
	8	43												
	14	69	37											
	15	42	39	47										
	25	6	12	10	9									
	30	24	20	20	25	8								
	32	18	12	22	8	2	15							
LA2	6	**9***	**11***	**18***	**10***	**9***	**1***	**0.2***						
	7	**11***	**1***	**12***	**4***	**2***	**0***	**0***	28					
	9	**5***	**5***	**14***	**10***	**9***	**0***	0	40	32				
	17	**1***	**1***	**3***	**6***	**0.2***	0.2	**0***	18	19	17			
	28	**4***	**4***	**3***	**13***	**10***	**0.6***	3	40	15	20	16		
	22	**3***	**12***	**9***	**4***	**0.6***	0	0.2	24	12	9	19	41	
Serum Number		2	8	14	15	25	30	32	6	7	9	17	28	22
		LA1							LA2					

bold type X^2> 5* negative association

Since there was an appreciable number of people who did not react to either LA1 or LA2 sera, we postulated a third allele which we could not yet identify in this system.

Further evidence for the existence of a shared antibody identifying the LA1 or LA2 antigens came from absorption studies of the sera in a group. From this work it was shown that our collection contained two pure sera, one for LA1 and another for LA2. The removal of the extra reactivities of a serum containing either LA1 or LA2 demonstrated that the sera were multispecific. Multispecificity has since been shown to be characteristic of the HLA system of antibodies.

A summary of the LA1, LA2 segregation in families is shown in Table 5. All but

Table 4. X^2 Relationships of 4b and putative 4a sera

Serum Number										
4b	3									
	10	28								
	11	19	36							
	18	11	34	35						
	19	4	35	29	56					
	26	2	4	3	11	26				
Putative 4a	4	**0***	**2***	**2***	**5***	**5***	**0.5***			
	31	**0.1***	**2***	**1***	**4***	**10***	**3***	1		
	33	**0.2***	**0.1***	**3***	**6***	**6***	**5***	0.5	9	
Serum Number		3	10	11	18	19	26	4	31	33
		4b						Putative 4a		

bold type X^2> 5* negative association

Table 5. Familial segregation of LA1 and LA2

Parental Types				No. of Families	Total Offspring Typed	Offspring Types			
						LA1 LA2	LA1 LA2	LA1 LA2	LA1 LA2
LA1	LA2	LA1	LA2			+ +	+ –	– +	– –
+	+	+	+	1	–	–	–	–	–
+	+	+	–	–	–	–	–	–	–
+	+	–	+	2	1	0	0	1	0
+	+	–	–	2	9	0	6	3	0
+	–	–	+	10	11	6	4	0	1
+	–	+	–	1	5	0	4	0	1
+	–	–	–	5	10	0	7	0	3
–	+	–	+	7	9	0	0	9	0
–	–	–	+	7	20	0	0	9	11
–	–	–	–	5	5	0	0	0	5

one of the ten possible mating types were observed. The segregation of the (+/–) x (–/–) and (–/+) x (–/–) matings indicated dominant inheritance of these antigens. The lack of any recombinants in LA1, LA2 x negative was expected on the assumption that the genes for LA1 and LA2 were allelic. The occurrence of (+/+) and (–/–) in (+/–) x (–/+) matings showed that the genes determining the antigens LA1 and LA2 were codominant, as is generally found for antigen markers. The population frequencies of the four types of reactions to anti-LA1 and anti-LA2 are shown in Table 6. Only data from unrelated individuals was included.

The definition of the LA system represented our first publication on white blood cell types in 1964. From then on we and others continued to expand the leukocyte antigen system. In 1964, the first international workshop was called. At this meeting we met to demonstrate the various techniques in use and to exchange data. Another workshop called the following year in Leiden continued these studies, particularly in comparing the behavior of the antisera. In 1965 Bruning and vanRood in summarizing the data on antigen types found that Mac, B1, LA2 and Group 2 of Terasaki and 8a appeared to be identical. LA1 was not found and it was assumed then that this must be a weak antigen. But in later studies, it proved

Table 6. LA phenotype and gene frequencies

Phynotype				
LA1	LA2	Observed	Expected	Gene Frequencies
+	+	11	12.3	(and standard errors)
+	–	29	27.8	LA1 = 0.1617 ± 0.03
–	+	54	53.0	LA2 = 0.2811 ± 0.023
–	–	41	41.9	LA3 = 0.5572 ± 0.033
Total		135	135.0	

Contingency X_1^2 testing (negative) association between LA1 and LA2 reactions = 9.7 (p <0.5%)
X_1^2 testing goodness of fit of expected phenotype frequencies = 0.227 (p >10%)

real. Quite a difference from today when there are currently defined 23 A-locus specificities, 50 B-locus specificities and 11 C-locus specificities in the Class I genes of the HLA System.

This description of my initial findings in this field was truly exciting for me. It led me among other facets to explore the role of the HLA antigens in transplantation. Since at Stanford we did not have such a program, I traveled up to San Francisco where Dr. Najarian had an active program in kidney transplantation in humans. I requested the opportunity to type his patients. But initially we had only seven antigens with which to work. As you currently know, the role of HLA antigens in kidney transplants was not clarified with that limited number of antigens. As I continued in research, my emphasis expanded primarily to the definition of new antigens. Participating in the International Workshops was a primary effort in that direction. That joint effort brought me new friends among Americans, Europeans, Africans and Asians. The work occupied me fully for several decades. Now it is time for new workers to solve the more complex problems on the character and significance of the polymorphism.

CURRICULUM VITAE

Rose O. Payne

PERSONAL

Born:	August 5, 1909

EDUCATION

1932	University of Washington, B.S. Bacteriology
1933	University of Washington, M.S. Bacteriology
1937	University of Washington, Ph.D. Bacteriology

TEACHING AND RESEARCH APPOINTMENTS

1934	Research Fellow, Woods Hole Oceanographic Institution, Massachusetts
1933-37	Teaching Assistant, Bacteriology, University of Washington
1937-38	Assistant Professor of Bacteriology, Oklahoma Agricultural and Mechanical College
1938-39	Research Fellow, McDermott Foundation for Study of Tuberculosis, University of Washington
1939-43	Lecturer in Bacteriology, Seattle College
1948-51	Research Assistant, Stanford University Hospital
1951-53	Research Assistant, Stanford University School of Medicine
1955-64	Research Associate, Stanford University School of Medicine
1964-72	Senior Scientist, Stanford University School of Medicine, Department of Medicine, Division of Hematology
1972-75	Professor of Medicine, Stanford University School of Medicine, Department of Medicine, Division of Hematology
1975-	Professor of Medicine, Emeritus, Stanford University School of Medicine, Department of Medicine, Division of Hematology

AWARDS AND HONORS

1964	John Elliott Memorial Award - American Association of Blood Banks, for research in leukocyte immunology
1977	Karl Landsteiner Memorial Award - AABB
1978	Tentative Society Award
1980	Katherine D. McCormick Distinguished Lecture

CURRENT PROFESSIONAL RESPONSIBILITIES

1970-	Member WHO Expert Advisory Panel on Immunology
1967-	Member International Committee on Nomenclature for HL-A Antigens
1970-	Member Committee on Organ Transplantation and Tissue Typing for American Association of Blood Banks
1964-	Councillor - Histocompatibility Workshop Conference

Now
admit it Rose,
I was first

Rose Payne, Award luncheon 1988

Judy Falk	Edmund Yunis	Rose Payne	Paul Terasaki	Nancy Goeken

The HLA Story as seen from Leiden

J.J. van Rood and A. van Leeuwen

Address: Dept. Immunohematology and Blood Bank
Building 1, E3-Q
University Hospital
P.O. Box 9600
2300 RC Leiden
The Netherlands

J.J. van Rood **A. van Leeuwen**

1966

1988

THE HLA STORY AS SEEN FROM LEIDEN

J.J. VAN ROOD AND A. VAN LEEUWEN

HLA is now over 25 years old and it seems to be a good time to look back at the times that it all began. Doing so one can easily discern three periods: the first concerned the pre-HLA period, the second the discovery of HLA and the third the HLA workshops.

The first period started in 1900 when Landsteiner (1) discovered the ABO blood groups and with that, immunogenetics, the art of studying genetically determined polymorphisms with immunological techniques. In the years that followed the MNS, P, Le and later the Rhesus blood groups were discovered. They were all *independent* systems which were expressed on erythrocytes. Landsteiner considered the possibility that they were transplantation antigens but did not pursue this further.

If Landsteiner is the father of immunogenetics, Gorer (2) earns that title for the histocompatibility antigens. He introduced terms such as tissue and transplantation antigens which he studied using a tumor system in mice. H-1, H-2 and many others were identified by him. The serological tests were mainly done using erythrocytes as target cells, which is possible because H-2 can easily be detected by erythrocyte agglutination. We know now that HLA antigens can also be detected on reticulocytes and very young erythrocytes but this is not how HLA was discovered. Much later we tried together with Pablo Rubinstein to set up a reticulocyte agglutination technique, but that never became operational (3).

Leucocytes as carriers of the transplantation antigens were identified as such by Medawar (4) in the forties. Amos showed that the H-2 antigens could also be identified by leucocyte agglutination (5). While this work was going on in animals, leucocyte serology had also started in man, not to detect alloantibodies but autoantibodies, especially in drug induced cytopenias. This field had become quite popular in the fifties, mainly through the beautiful systematic work of Ackroyd (6) on quinidine-induced thrombocytopenia. Moeschlin (7) detected leucocyte agglutinins in patients suffering from granulocytopenia due to pyramidon. His method was soon modified to a leucoagglutination assay using buffy coat cells separated from defibrinated blood by dextran sedimentation. Reproducibility was poor at about 70%. In Finland, Wasastjerna (8) introduced EDTA as an anticoagulant and Van Loghem and his coworkers, especially Engelfriet, improved and popularized that method (9). Reproducibility of this method was over 80%.

When it was realized that many of these so called autoimmune antibodies were in fact alloimmune antibodies, many teams joined the fun, our group being one of them. It took the better part of the fifties to establish the existence of alloantigens on leucocytes. A few observations really helped. Miescher (10) described an antibody in the serum of a multitransfused woman which agglutinated some but not all leucocytes of a panel consisting of 22 donors. This was 1954. In the same year Dausset (11) showed that leucoagglutinins appeared in the blood of multitransfused patients. One would think that this would clinch the matter but nothing is less true.

People remained confused and in the same year a paper was published in which it was speculated that the leucoagglutinins present in the serum of patients with aplastic anaemia might be autoimmune antibodies and causative in the disease. Although it was thus realized that there must be something like leucocyte groups, the tools to recognize them were lacking.

Then there was another important point. The idea that there would be one major histocompatibility complex was nonexistent. After all, our thinking had been strongly influenced by the discovery of the blood groups, of which there were many independent systems. Interest of the blood banking community in leucocyte serology was activated when Van Loghem (12) discovered that leucoagglutinins could cause non-hemolytic blood transfusion reactions.

Dausset, using leucoagglutinins formed after planned blood transfusions, described 4 sera which on a panel of 25 cells seemed to recognize the same antigen which he called Mac (13). The difficulty with the Mac antisera was that they were weak and soon lost their activity. As a result many workers in the field remained skeptical.

The problem was solved when virtually at the same time, but independently, it was realised in Leiden by our group (14) and in Palo Alto by Rose Payne (15) that pregnancy per se induced leucoagglutinins as well.

We stumbled on the fact by serendipity, Rose Payne by beautiful systematic work. It was a clinical observation, which made us realise that pregnancy could induce leucoagglutinins. On April 14, 1958 Mrs. H., a mother of four children, was delivered of twins. Soon afterwards she had a postpartum bleeding which led to preshock and she was transfused. The blood pressure rose again but a few hours later the patient had shaking chills, felt nauseated and collapsed. Thanks to Van Loghem's findings, we realized that the patient probably had suffered from a non-hemolytic transfusion reaction caused by leucoagglutinins. Strong leucoagglutinins were indeed found, but that in itself confronted us with a question. The question being where and when the patient had received the blood transfusions. Blood transfusions were up to that moment thought to be the only inducers of leucocyte antibody formation. Actually a report had been published that in a hundred women with previous pregnancies no antibodies had been found. Leiden is a small town, the patient was born in Leiden and lived there and George Eernisse could not find in the Blood Bank a record of previous transfusions. When we went back to the patient to ask where she had received these blood transfusions she looked at us in horror and said: "This was the first blood transfusion I have ever received and as far as I am concerned, it will be the last."

Only then did we realise that the previous reports might be wrong and pregnancy per se could induce leucocyte agglutinins. For some still unknown reasons such agglutinins remained stable for many years after delivery. One of our best sera produced by the wife of a butcher from a nearby village was found 35 years after her last delivery!

It was with such sera that the first family studies were carried out. The groups were given numbers. The serum detecting group 1 was the one from Mrs. H, who had opened our eyes. The sera for groups 2 and 3 were both oligospecific, a term coined by Walford to indicate that – although one could never be one hundred percent certain – the antibody was truly monospecific, by cross-absorption experiments one could make a pretty good guess that there were at least not many different specificities in a serum.

These data were published and also presented in Rome in 1958 (16) during the hematology and blood transfusion meeting. In the same meeting Dausset presented his data on Mac. The meeting was memorable for other reasons as well.

We were of course newcomers in the world of white cell serology, but we knew quite a few people in the blood banking world. One of them was Dr. James, head of the Northern Blood Transfusion Service UK, a gigantic operation and Dr. James as the director looked and acted the part. When we told him about our leucocyte antibodies induced by pregnancy and especially when we told him that these could be absorbed by platelets, he looked at us with a knowing smile and said: "Well, I don't know but you might have to eat your hat" (an expression we had not heard before but it was quite clear that Dr. James did not believe us). This skepticism was at that time general. That same morning we met Dausset and Colombani on the steps leading to the congress building and they were arguing about the same point: are platelets able to absorb leucoagglutinins. When Dausset heard that we had found the same, he turned to Colombani and said:" What did I say! Are you now convinced!"

One could tell many similar anecdotes all reflecting how little we knew and how uncertain we were about our findings. This was certainly, to no small measure, due to the poor reproducibility of our techniques. As I said, 70% for the defibrinated agglutination technique most people were using.

An important technical improvement was therefore the realisation that although a serum could not agglutinate a leucocyte sample of a certain donor, these same leucocytes were sometimes able to absorb the antibody out of the serum. This was called the ANAP phenomenon (agglutination negative absorption positive). Later also called CYNAP for the cytotoxity assays. That serum, nr. 36, later turned out to be the first of a cluster of sera which recognised the same or at least very similar antigens. We called it antigen Four which is now called Bw4.

And now things really started to move and we are entering the second period of the HLA story. We had good antibodies, a reasonable technique, the EDTA technique which was much improved because we had recognized the ANAP (or CYNAP) phenomenon. So we set out to do what was at that time considered a very large experiment: 60 sera from women who had been pregnant and which contained leucocyte agglutinins, were tested against the leucocytes obtained from a hundred random panel donors.

This is now a standard procedure which still forms an essential part of all workshops, but at that time (1960-1961) something like that had never been done. It took several weeks to do all the tests (Fig. 1). The only way in which we could analyse the data was by comparing positive and negative reactions obtained by each serum against the positive and negative reactions of all other sera and then to do a two by two chi square test (17). The idea to do it this way was born one day when following a course on medical statistics. We realized that if two sera would show a significant association even if they did not react identically (even after taking ANAP into account), it still meant that they must have something in common and that that something could be an antigen which they both recognized. All rather obvious now, but at that time it was new at least for us. Doing such an analysis by hand would be next to impossible: we would have to do 3600 comparisons. Thus we decided to find a computer to do the work for us. At that time the Medical Faculty had not one single computer. The only one the Leiden University owned, was at the Department of Astronomy for the calculations of distances, speeds etc. That machine was completely useless for the kind of analysis we wanted to do. By chance we heard about a firm in Utrecht and went there. On a big attic they had an enormous "cuckoo-clock", a mechanical Hollerith sorting system, all wooden hammers and wheels. They were very nice people who really understood what we were talking about. A week later they came with the analysis and they were proud. It was fantastic, they had really understood everything we had said. The only thing wrong was that they had not compared every serum with every serum, but every cell with every cell! Quite a number

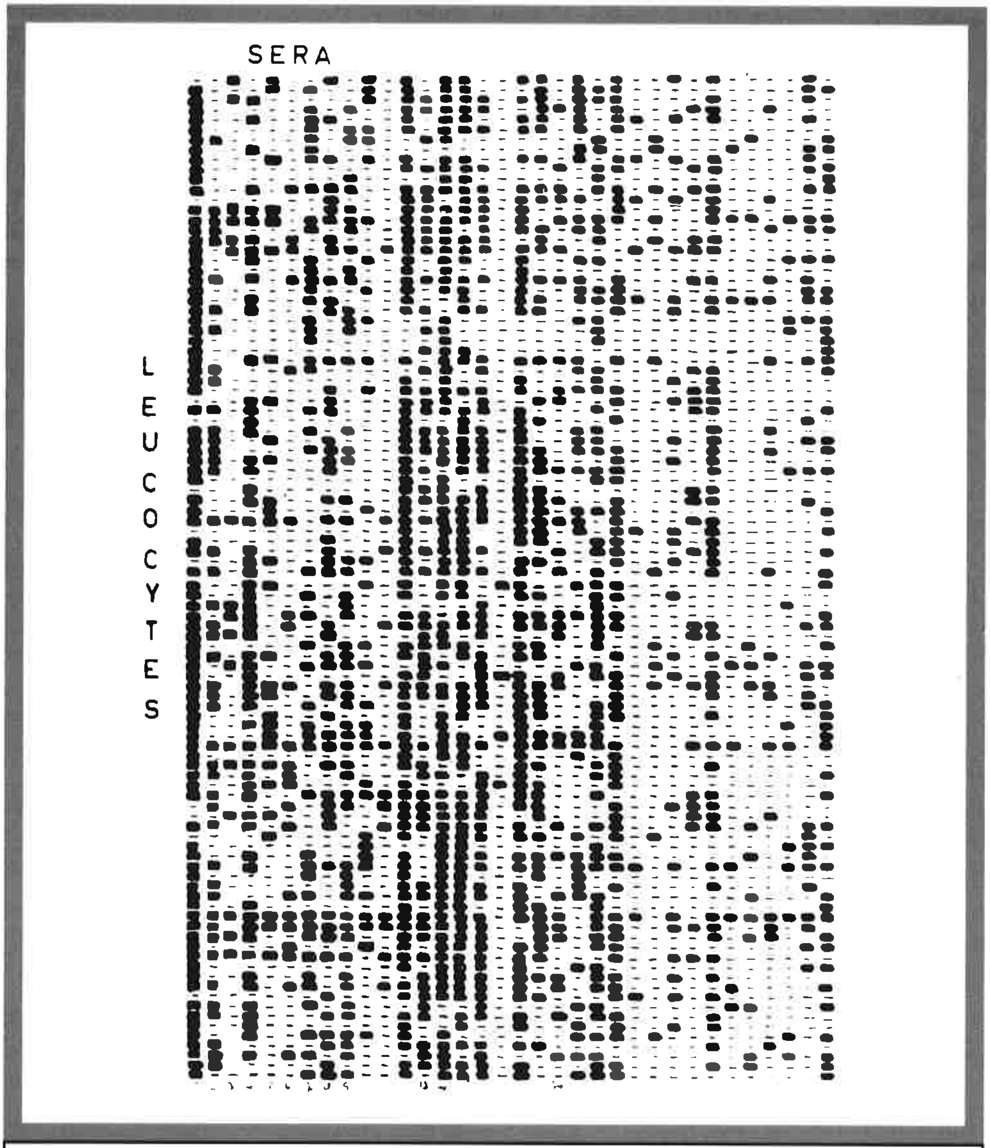


Figure 1. Agglutination pattern of 34 sera with leucocyte antibodies tested against 100 leucocyte samples. Every vertical row of squares and hyphens shows the results obtained with one serum against the panel; every horizontal row shows the results of the 34 sera with one leucocyte sample. Black squares mean agglutination positive; hyphen means agglutination negative. Doubtful results were recorded as agglutination negative. (from J.J. van Rood 1966)

of very interesting patterns came out but it was too early for us to interpret them. After that they went bankrupt and we have never known whether this was because they had done these experiments for us or whether it was due to some other reason. I must confess we did feel a bit guilty.

Anyhow the search went on and of course we came in contact with IBM. A very nice young man in a tailor made suit came to the department and we explained what we wanted. "No problem, IBM can handle that", he said and disappeared. Three weeks later he came back with some "minor" questions he wanted to clarify. It was clear that he had not understood a word of what we had been talking about and he suggested, and we agreed, that he would come back with one of the programmers. That man, an elderly man, became very excited. He understood immediately what we were saying and answered: "Of course, that is something special for our 104". At that moment someone kicked my shine-bone rather painfully.

Later, I found out that the computer 104 (the only computer able to handle our analysis at that time) had been sold (and not lent or borrowed) to the Dutch Bureau of National Statistics. So IBM could not help us (the kick on my shine-bone was not meant for me but for the programmer) and lost a client. But the Bureau of National Statistics was able to do the job and together with the department which was responsible for calculating the salaries of the Dutch government's employees we got our first really good analysis. The pattern which came out is shown in Figure 2. A very nice cluster which we named 4a and 4b, Bw4 and Bw6 now. All this happened in 1961.

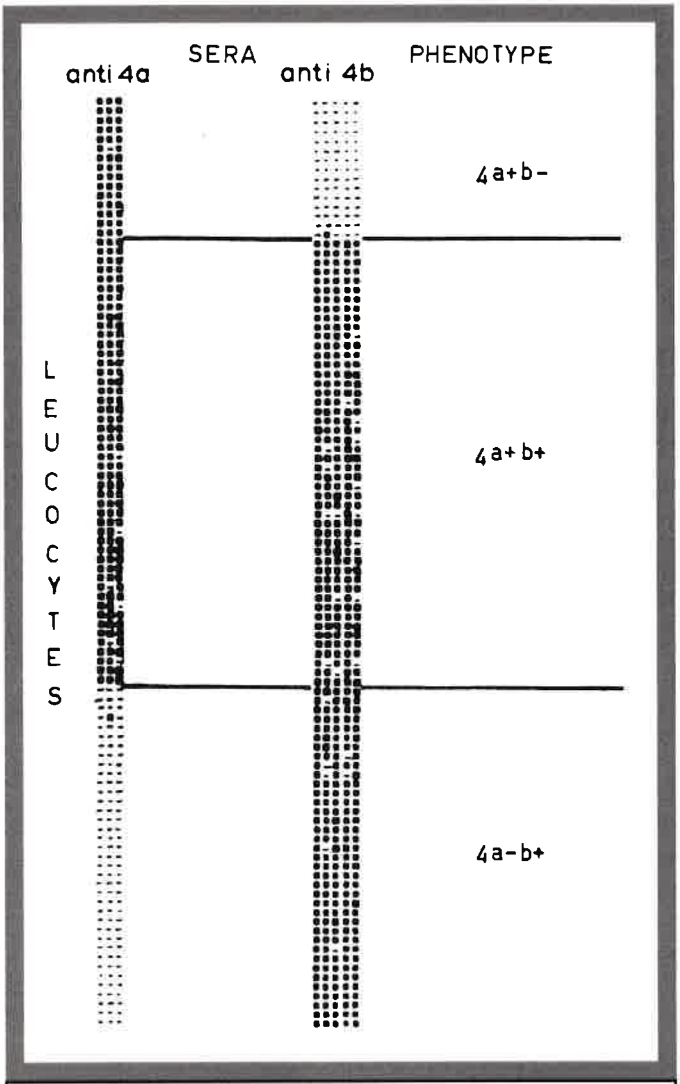


Figure 2. Agglutination pattern of 8 sera with the leucocytes of the panel. Every vertical row of squares and hyphens shows the results obtained with one serum against the panel; every horizontal row shows the results of the 8 sera with one leucocyte sample. Black squares mean agglutination positive; hyphen means agglutination negative. Doubtful results were recorded as agglutination negative. (from J.J. van Rood 1961)

At the end of 1961 we went to Vienna to present our data (18); Dausset was there as well and was quite interested in our findings. We were pleased as Punch that he was so generous in his praise.

Not everybody was convinced however and to be absolutely certain we repeated the testing of the 60 sera against the whole panel using both the defibrinated and the EDTA technique. We, in this case, were not only Aad van Leeuwen but also Ali Schippers. The results came out exactly the same, the EDTA results being the cleanest. Our studies were put together in 1962 in a thesis: "Leucocyte Grouping, a Method and its Application.", a title suggested to me by my friend and colleague Fred Loeliger, the coagulation expert. In Holland a thesis is really a printed book and we proudly sent it to all the workers in the field (17,19). Rose Payne and Walter Bodmer were the first to introduce this computer approach in their laboratory. A few months later we got a letter from Rose Payne saying: "Dear Jon, could you send us another copy of your thesis. The copy you had sent to me has been travelling so often between Walter's lab and mine that it is completely hanging to pieces and we would like to have a new one." We sent it immediately. In 1964 they described the LA1, LA2 and LA32 antigens (20). It was (and still is) of interest that they, using the defibrinated leucoagglutination technique, identified the HLA-A locus antigens while we, using the EDTA leucoagglutination technique, identified the Four locus antigens. Might CD8 have something to do with this?

In that same period two important technical improvements were realized. The first was the introduction of the complement dependent cytotoxicity assay, as it had been used in the mouse for more than a decade by several people but notably by Batchelor who had actually been trained by Gorer. The second was the development of the microcytotoxicity test by Terasaki. A truly revolutionary technical improvement. If you were very good you could do 20-25 agglutination tests with one ml of serum, but with the microcytotoxicity test you could do a thousand (21).

It was Bernard Amos who took the initiative for the first workshop. The first meeting in 1964 was in the National Academy in Washington, the workshop in Duke University, North Carolina. It centred mainly around a comparison of techniques. I was asked to summarize the meeting. A hopeless task because everybody had used different cells, different sera and different techniques. The only thing I could think of was to invite everybody to Leiden where we had a panel typed for 7 well-defined antigens. The 1965 workshop set thus the format which was followed during all the subsequent workshops; a panel of cells was tested by the different teams using their own sera and techniques.

The results were most gratifying and it was decided to continue the workshops. The 1965 workshop in Leiden was also a turning point because during this meeting family data from our group and population data from Dausset provided strong evidence that most of the antigens recognized belonged to one genetic system. We had called it the group Four system, Payne and Bodmer LA and Dausset Hu. After an emotional meeting in Williamsburg we dropped group Four and HLA was born.

There is another reason why the 1965 workshop is memorable and that concerns the demonstration that the HLA antigens are transplantation antigens. The protocol was simple (22). All we wanted to do was to repeat the skin transplant experiments of Medawar in man. This was possible thanks to Felix Rapaport who had developed a very nice skin grafting technique. We of course needed volunteers. Many of our colleagues who helped us with building the big panel by which 4a and 4b had been identified, were approached by us and got the shock of their life when we asked them whether they would be willing to give and/or to receive a

Table I. (from J.J. van Rood et al. 1965, ref. 22)

Survival Time of 6^b6^c Compatible and Incompatible Skin Grafts in Recipients Preimmunized against 6^b6^c Antigens

Immunizing dose (i.d.)	Interval	Survival of skin graft	
		$6^{b+}6^{c+}$	$6^{b-}6^{c-}$
12×10^7 leuk.	14	0	12
22×10^7	18	5	9*
20×10^7	16	5.5	8.5
5×10^7	13	7	14
20×10^7	14	9	11
21×10^7	14	9.5	11.5
	Mean	6	11

* This recipient was $6^{b+\,c-}$

skin graft. George Eernisse and I set the example and they agreed to collaborate. This was long before the days of the medical ethical committees! We had worked out the protocol with Balner in monkeys and had shown that it worked (23).

We injected 2×10^8 leucocytes incompatible for one antigen only (6^b6^c, now called B7) with the recipient intradermally and two weeks later a skin graft of a leucocyte donor, which was B7 negative and one which was B7 positive, was transplanted. Table I gives the skin graft survival data in man for HLA-B7, formally proving that HLA antigens were transplantation antigens because they influenced graft survival (22).

An important contribution of Ceppellini was to combine family studies with skin graft experiments. After the workshop we travelled to Torino to perform the HLA typing of skin donors and recipients and could show that also in non-immunized recipients graft survival correlated with HLA matching (Fig. 3) (24). Matching for the independent Group Five system apparently had no influence. Amos made a similar observation almost at the same time.

From this kind of data followed our studies on the kidney survival (25, 26), which eventually led to the proposal and foundation of an international organ exchange organization: Eurotransplant. And this was the end of the second era.

We were not only aware that there was something worthwhile to detect but we also had the tools and know – how to do the job and we knew what we were looking for.

The third era had begun and it was the era of the workshops. Their contributions were many and here again we can mention only a few highlights. Ruggero Ceppellini, who (together with Walter Bodmer) gets the credit of educating the HLA community in the basics of genetics. He has made a number of very important contributions in the field of immunogenetics, of science in general, including immunology, and he has been in many ways the brain behind many of our experiments. He also had a very strong personality. He organized the third meeting, the Torino meeting in 1967. I was – having organized the previous one – the president and we had some "open hearted" discussions when he was complaining about the misbehaviour of the participants of the meeting who were drinking about three times the average amount of wine the Italians used to drink! They then had a hangover the next day and had all kinds of complaints when the laboratory work had to be done.

It had been decided to do family studies. Ceppellini suggested that it would be fun to have some duplicates, and set out to select families with monozygotic twins. When the data were analyzed, it turned out that with some of the techniques, especially the very sensitive ones, it was impossible to differentiate between monozygotic twins and dizygotic twins, let alone a parent from a child.

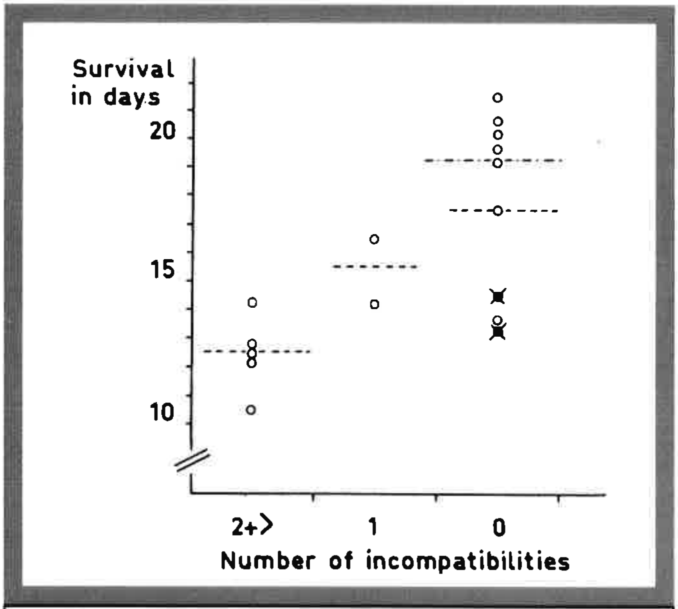


Figure 3. Relation between skin graft survival and ABO- and leucocyte-group compatibility. Donor and recipient were sibs.
------ = indicates mean survival time.
⊗ These recipient-donor combinations were incompatible for the antigens recognized by the cytotoxic sera.
If these incompatibilities are not taken into account, the mean survival time is indicated as -·-·-
(from J.J. van Rood et al. 1966, ref. 24)

Ceppellini with Mattiuz, Piazza and Bodmer was the one who introduced the concept of linkage disequilibrium. He was also instrumental together with Miggiano and Lightbody in introducing the CML test, the cell mediated lympholysis test (27). Lightbody presented the data for the first time in Paris and when asked why he called this test the CML test, said: "Well, officially we say it is the cell mediated lympholysis test, but you of course understand that it stands for Ceppellini, Miggiano and Lightbody".

There are at least three other things we would like to mention. One is the birth of the MLC test. Again many, many stories prevail. Fritz Bach (28) and Barbara Bain (29) discovered independently the MLC test at about the same time. Bach by overhearing a discussion between Jim Gowans, a famous scientist in England, and Peter Medawar, in which they discussed in 1963 the fact that lymphocytes appear to carry transplantation antigens because they could induce homograft sensitivity, and that they could also form blasts and could divide. Fritz thought: "Well if lymphocytes can do that, why not mix cells of two individuals and see what happens. The two way MLC test was born, later to be refined by Ceppellini to the one way MLC test. Barbara Bain stumbled on the MLC test in a serendipity fashion. By mistake cells from two individuals were mixed in a tube and the PHA was forgotten but they still got blasts and she realized what she had found. She really did much better than Dr. Schreck who in 1962 published a paper in which he mentioned in the discussion that by mistake two samples had been mixed and had formed blasts but because of that he had left out the results.

The MLC test made it possible to type for HLA-D. Bradley (30) was the first to do HTC typing in pigs. Unaware of Bradley's paper we reinvented the wheel and were looking for homozygous typing cells to be used for HLA-D typing. We realized that they could best be found in cousin marriages' offspring. The problem obviously was how to identify such people. Thanks to one of the Ph.D. students, we were aware of the fact that the Catholic Church does not allow cousin marriages unless the Pope has given permission and of course the Catholic church keeps excellent archives on these cousin marriages. We wrote a letter to the Pope asking for his permission to use that information. Sure enough after a few months we received a letter back that we should contact the bishop of Haarlem. We went there to discuss the problem, got his blessing and went ahead. (31)

Dausset heard the story and said: "What can be done in Holland, should certainly be possible in France", and he went to the Evèque de Paris, explained his case but the Evèque de Paris said: "Non" and that was the end of it! To solve the problem Degos and Colombani went to the Toureggs who must marry their cousins. Unfortunately (and interestingly) they found far less homozygous children than expected. Is this due to chance, lethal genes (W. Bodmer) or diminished resistance against infections? All possible, but none proven.

After Torino the Los Angeles (1970) and Evian (1972) workshops took place reinforcing the importance of HLA for organ transplantation (the very significant difference for HLA identical sibling versus parent-child versus unrelated transplants) and a first attempt "to HLA map" the world. In Evian Eijsvoogel presented his beautiful data on a family with a crossover between HLA-D and HLA-B and in another between HLA-B and HLA-A. Doing CML studies he could show that one needed both an HLA-D and a class I disparity to get a positive CML test (32).

While the recognition of the HLA-A and -B antigens could be systematically worked out since 1962, it took another ten years and partly new methodology to attain the same for HLA-DR. This was not only due to the fact that it took several years before it was recognized that the determinants which stimulated in the MLC were not identical to HLA-A and -B (as originally assumed), but also because it took even longer before it was accepted that the HLA-D locus was polymorphic like the HLA-A and -B loci and methods to recognize this polymorphism became available. As a matter of fact the analysis of HLA-D and DR took place simultaneously.

Much of the pressure to search for methods to type for HLA-D and especially DR came from those involved in clinical organ transplantation. It had already been recognized that low or negative MLC reactivity improved graft survival of both skin and kidneys in man and (for the kidneys) also in monkeys. It was clear that one way of selecting MLC negative (or weakly reacting) donor-recipient pairs for kidney transplantation when postmortal donors had to be used was to be able to type them for HLA-D, i.e. the system responsible for the strongest stimulation in the MLC test. Although typing with homozygous typing cells made typing for HLA-D, it was too time consuming to be useful in clinical renal transplantation using cadaveric donors. A serological method would be the solution if such a method could be developed. As a matter of fact there existed in the beginning considerable scepsis that this would be feasible. Much of this scepticism was based on the fact that in many other systems T cell dependent immunity differed in its specificity and antigenic requirements from B cell dependent immunity. (Erna Möller: a very personal communication)

That we decided to study the problem of HLA-D related serology anyhow, was in fact due to two observations one of which was made by Ceppellini and his coworkers and one by us. Ceppellini had in a large study shown that sera with anti-HLA-A and -B antibodies were able to inhibit the MLC test. Although MLC inhibition by HLA antibodies was thus a well established phenomenon, the mechanism by which this occurred was poorly understood. Again serendipity opened our eyes. A woman, mother of two children, had received an

Table II. Typing for MLC LD: MLC Inhibition and Immunofluorescence (from A. van Leeuwen et al. 1973, ref.33)

Parous Woman Sch: HL-A2,3,7, W10				
	AB Serum (c.p.m.)	Serum Sch (c.p.m.)	AB Serum/ Serum Sch	Fluorescent (% Pos. Cells)
Sch + P_m	3,300	400	*7.3*	*17*
Q_m	14,000	8,000	1.7	4
N_m	9,700	1,100	*9.5*	*17*
R_m	10,000	6,400	1.6	9
S_m	4,200	2,800	1.7	7
T_m	5,200	700	*7.4*	7
U_m	13,500	1,300	*10.0*	*16*
V_m	26,500	1,100	*24.1*	*17*
W_m	1,800	200	*10.0*	–

All stimulator cells were SD-identical with the responder cells Sch: HL-A2,3,7,W10. The column AB Serum/Serum Sch depicts the inhibition index values obtained by dividing the c.p.m. obtained by culturing the cells in AB serum by the c.p.m. obtained when they were cultured in serum Sch. The significant inhibitions are in italics.

MLC positive but HLA-A and -B identical experimental skin graft. On the basis of previous experience we had expected it to survive 15 days at most, but at 23 days it was still not rejected. We wondered whether there might be "something" (an antibody induced by pregnancy?) that neutralised the rejection inducing effect of an HLA-D mismatch.

We decided to use the MLC inhibition technique to screen for anti-HLA-D (now DR) antibodies. It was clear from the beginning that the test system had to be rigorously controlled to be certain that irrelevant antibodies (i.e. other than anti HLA-DR) could not cause MLC inhibition. To attain this we used responder cells which were obtained from the person – in most cases women with a history of multiple pregnancies – who had formed leucocyte antibodies. To exclude interference with anti-HLA-A and -B antibodies stimulator cells were used which were HLA-A and -B identical with the responder cells. We named this approach the MLC inhibition test using SD identical stimulator cells or MISIS for short. The sera which were tested contained strong anti-HLA-A and/or -B antibodies. This was done on the assumption that persons who were able to produce strong anti-HLA-A and/or -B antibodies might likewise also be able to produce anti-HLA-DR antibodies. An assumption which later was shown to be correct. The very first serum which was tested in the MISIS test was informative (Table II) (33). Some responder-stimulator cell combinations were inhibited (P, N, T, U, V, W) while others were not (Q, R, S). This finding was reproducible and other sera which showed similar results were found easily.

Next we asked the question whether the MLC inhibition was really due to an antibody. This seemed likely because the MLC inhibiting activity could be absorbed by stimulator cells which were inhibited.

More direct evidence that antibody mediated the MLC inhibition was obtained when Aad van Leeuwen together with Riek Schuit developed a sandwich immunofluorescence test with anti-IgG which gave an at that time unusually low background with normal lymphocytes. While the background was 4-8% some of the stimulator cells when tested with the serum used in the MISIS test showed bright staining of 16-17% of the cells (Table II). The low background immunofluorescence was clearly essential to detect these small differences and was originally dependent on very rare and very pure rabbit anti-human Ig sera which had been made by Jiri Ràdl in the Laboratory of Experimental Gerontology-TNO, in Rijswijk. When it was understood that the background staining was due to binding of fluorescein labelled immunoglobulins by Fc receptor molecules, commercially available sera were subjected to pepsine treatment to remove the Fc part of the anti-human Ig and the test became generally available.

As is illustrated in Table II, the first serum to detect polymorphism in the MISIS test showed also significantly positive fluorescence. The results of the two tests correlated quite well, a finding which was substantiated in later studies. The MLC inhibiting substance remained stable for years. It was assumed that the MLC inhibiting substance and the antibody which reacted in the immunofluorescence test were probably the same and thus an IgG molecule.

The next question which had to be answered was why only such a small percentage of the cells of peripheral blood reacted with these antibodies. This turned out to be due to the fact that these antibodies reacted with a determinant mainly present on B cells and monocytes but absent from (most) T cells and all platelets. The latter observation had practical consequences because it provided a simple possibility of purifying the anti-DR reagents: platelets lack DR but carry HLA-A, -B and-C antigens, while retaining the anti-HLA-DR antibodies.

When this information became available it was logical to test the antibodies found in the MISIS and/or immunofluorescence tests by complement dependent cytotoxicity. Early attempts failed because in man the number of B cells in peripheral blood is low, but when B cell enriched cell suspensions became available, also thanks to the help of Bob Winchester (34) through depletion of T cells by rosetting with sheep red blood cells, this problem was easily overcome and it could be shown that many of the sera contained complement dependent cytotoxic antibodies which reacted with B cells, and monocytes. Prolonged incubation times increased the efficacy of the long B cell or rosetting technique further. The test was standardized and with minor alterations used during the 7th Histocompatibility Workshop (35).

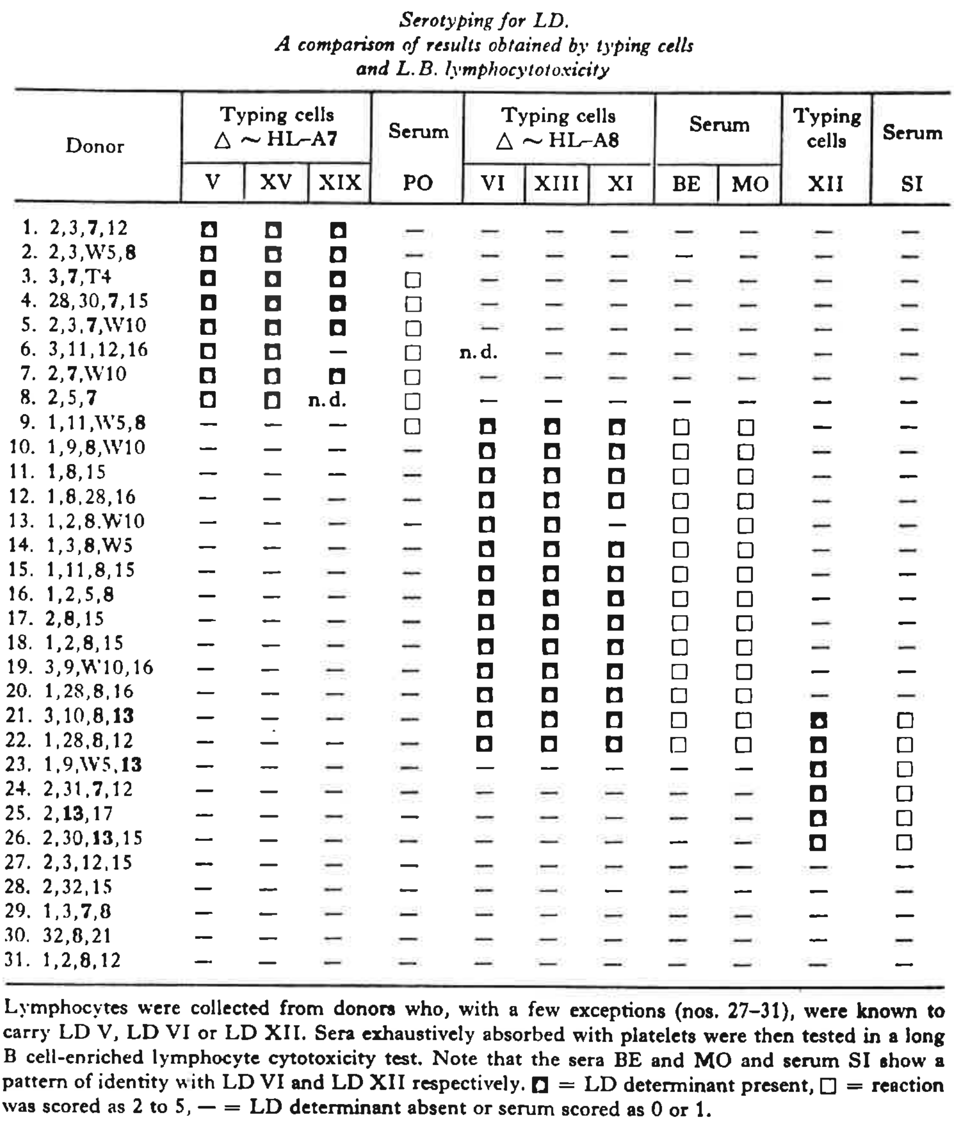
Although the rosetting B cell test is quite adequate when investigating healthy individuals, the B cell enrichment is tedious and with the blood of patients suffering from some diseases, quite difficult. To circumvent this obstacle a further refinement was introduced. Instead of enriching the B cells in the test cell suspension, they were identified with FITC labelled sheep anti-human Ig. The monocytes stained as well. Next a standard two stage complement dependent lymphocytoxicity test was carried out and the dead cells were identified by ethidium bromide. The red fluorescence of the latter stain and the green of the first can be read simultaneously by appropriate selection of (blocking) filters (36).

With these two requirements the detection of the HLA-DR antigens had been fulfilled: adequate techniques and reasonably pure antibodies were available.

The genetics of the system offered not too much of a problem either, because it was assumed from the beginning that HLA-D and -DR were very closely related to each other, if not identical. (DR stands for D related as decided after the 7th Workshop by Ruggero Ceppellini, Walter Bodmer and myself).

While the techniques were developed for HLA-DR typing as described above, the study of HLA-D using homozygous typing cells had progressed quite rapidly. Our

Table III. (from J.J. van Rood et al. 1975, ref. 35)



Serotyping for LD.
A comparison of results obtained by typing cells and L.B. lymphocytotoxicity

Donor	Typing cells Δ ~ HL-A7			Serum	Typing cells Δ ~ HL-A8			Serum		Typing cells	Serum
	V	XV	XIX	PO	VI	XIII	XI	BE	MO	XII	SI
1. 2,3,**7**,12	■	■	■	—	—	—	—	—	—	—	—
2. 2,3,W5,**8**	■	■	■	—	—	—	—	—	—	—	—
3. 3,**7**,T4	■	■	■	□	—	—	—	—	—	—	—
4. 28,30,**7**,15	■	■	■	□	—	—	—	—	—	—	—
5. 2,3,**7**,W10	■	■	■	□	—	—	—	—	—	—	—
6. 3,11,12,16	■	■	—	□	n.d.	—	—	—	—	—	—
7. 2,**7**,W10	■	■	■	□	—	—	—	—	—	—	—
8. 2,5,**7**	■	■	n.d.	□	—	—	—	—	—	—	—
9. 1,11,W5,**8**	—	—	—	□	■	■	■	□	□	—	—
10. 1,9,**8**,W10	—	—	—	—	■	■	■	□	□	—	—
11. 1,**8**,15	—	—	—	—	■	■	■	□	□	—	—
12. 1,**8**,28,16	—	—	—	—	■	■	■	□	□	—	—
13. 1,2,**8**,W10	—	—	—	—	■	■	—	□	□	—	—
14. 1,3,**8**,W5	—	—	—	—	■	■	■	□	□	—	—
15. 1,11,**8**,15	—	—	—	—	■	■	■	□	□	—	—
16. 1,2,5,**8**	—	—	—	—	■	■	■	□	□	—	—
17. 2,**8**,15	—	—	—	—	■	■	■	□	□	—	—
18. 1,2,**8**,15	—	—	—	—	■	■	■	□	□	—	—
19. 3,9,W10,16	—	—	—	—	■	■	■	□	□	—	—
20. 1,28,**8**,16	—	—	—	—	■	■	■	□	□	—	—
21. 3,10,**8**,**13**	—	—	—	—	■	■	■	□	□	■	□
22. 1,28,**8**,12	—	—	—	—	■	■	■	□	□	■	□
23. 1,9,W5,**13**	—	—	—	—	—	—	—	—	—	■	□
24. 2,31,**7**,12	—	—	—	—	—	—	—	—	—	■	□
25. 2,**13**,17	—	—	—	—	—	—	—	—	—	■	□
26. 2,30,**13**,15	—	—	—	—	—	—	—	—	—	■	□
27. 2,3,12,15	—	—	—	—	—	—	—	—	—	—	—
28. 2,32,15	—	—	—	—	—	—	—	—	—	—	—
29. 1,3,**7**,**8**	—	—	—	—	—	—	—	—	—	—	—
30. 32,**8**,21	—	—	—	—	—	—	—	—	—	—	—
31. 1,2,**8**,12	—	—	—	—	—	—	—	—	—	—	—

Lymphocytes were collected from donors who, with a few exceptions (nos. 27–31), were known to carry LD V, LD VI or LD XII. Sera exhaustively absorbed with platelets were then tested in a long B cell-enriched lymphocyte cytotoxicity test. Note that the sera BE and MO and serum SI show a pattern of identity with LD VI and LD XII respectively. ■ = LD determinant present, □ = reaction was scored as 2 to 5, — = LD determinant absent or serum scored as 0 or 1.

group was especially fortunate because Keuning, Termijtelen et al. had typed a well-sized panel and a number of families for HLA-D with homozygous typing cells, which facilitated the comparison of the distribution of HLA-D versus HLA-DR enormously (37). The results showed that in the Dutch Caucasian population the distribution of HLA-D and -DR was very similar. (Table III)

We are writing now 1975, the year of the Aarhus Workshop hosted by Flemming Kissmeyer-Nielsen and old time friend. Flemming has been in many ways an important figure and contributor to the HLA field in general and to our group in particular. Being the first to identify and recognize as such a crossover between HLA-A and -B, he helped us in setting up the microcytotoxicity technique. Using that technique was an essential cornerstone of the whole Eurotransplant organization: the cells could be easily shipped and stored and of course a thousand tests per ml is good mileage. He and Erik Thorsby provided many of the sera used in Eurotransplant. At the tenth anniversary of Eurotransplant I wrote them a note thanking them for their help while noticing that "many of our organ exchanges would have been blind dates" without them! And so one could go on, but we will not. The year 1975 seems a good point to stop: HLA was well established with many people entering the field and wanting to write their own story. Writing this chapter has been fun but also frustrating. With every anecdote, every "breakthrough" told, one realises how much one does not record. The good advice of Sylvia Lawler to do family studies; Willy Hijmans leading us through the intracacies of the fluorescence microscope; and Roy Walford into yoga, ageing and immunology; Parviz Lalezari, one of the few who was, like us, also interested in non-MHC antigens; Pablo Rubinstein, Hans Balner, Hans Bruning and so many others who taught us this or that and then corrected us when we made a mistake. We apologizes to them and all the others we have not mentioned. The HLA field was and is fun, mainly because it implied working with them.

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CURRICULUM VITAE

Johannes Joseph van Rood

PERSONAL

Date of birth: April 7, 1926
Nationality: Dutch
Place of birth: The Hague, The Netherlands
Married: Sacha Baronesse van Tuyll van Serooskerken
Children: Yanda, Peter and Tinka
Home address: Laan van Alkemade 3, Oegstgeest, The Netherlands
Office address: Department of Immunohaematology & Blood Bank,
Bldg. 1, E3-Q, University Hospital,
Rijnsburgerweg 10, 2333 AA Leiden, The Netherlands
Tel. No.: (0)71-263800/263827

MEDICAL TRAINING

1945-52	University of Leiden (medicine)
1950	Department of Medicine (Dr. R.F. Loeb), Presbyterian Hospital, Columbia University, New York, N.Y., U.S.A.

RESIDENT AND POSTGRADUATE TRAINING

1952-57	Department of Medicine, University Hospital, Leiden
1953	Training in blood banking, Central Laboratory of the Dutch Red Cross Blood Transfusion Service, Amsterdam
1957	Specialist in Internal Medicine (equivalent to US Board Examination). In charge of Blood Bank and Department of Immunohaematology, University Hospital, Leiden
1958-	Engaged in tissue typing
1962	Doctor of Medicine (cum laude) (equivalent to US Ph.D. or Doctor of Medical Science); thesis: "Leucocyte Grouping. A method and its application" (Prom.: Prof. Dr. J. Mulder).
1962-63	Sabbatical year in the Department of Immunology, Public Health Research Institute of the City of New York., N.Y., U.S.A. (head: Dr. F.L. Adler)
1981	Certificate "Immunology" of the Registry of Immunologists of the Dutch Society of Immunology
1986 (Feb-Apr)	Sabbatical, Department de Microbiologies C.M.U. Faculté de Médecine Genève

APPOINTMENTS

1957-	Head of the Department of Immunohaematology
1965	Lecturer in Internal Medicine
1967	Founder and President of the Eurotransplant Foundation
1969	Professor of Internal Medicine

1976	Chairman of the Department of Haematology
1985	Founder and President of the European Foundation of Immunogenetics
1986-	Guest Professor "Vrije Universiteit" Bruxelles
1986	Co-founder and Chairman of Leiden Institute for Immunology
1987	Supervisor for the training of Immunology postgraduate students

ADVISORY COMMITTEE MEMBERSHIP, EDITORIAL BOARD, ETC.

Advisor of the Primate Center TNO, Rijswijk, The Netherlands

Member of the "Working party on the exchange of sera for the determination of histocompatibility" of the Council of Europe

Member of the Royal Dutch Academy of Sciences (section Physics, since 1978)

Member of the International Scientific Council of the "Foundation Cardiologique Princesse Liliane"

Member of the Advisory Board of Tissue Antigens

Member of the Advisory Board of Transplantation Proceedings

Member of the Editorial Board J. Immunopharmacology & Immunotoxicology

Member of the Editorial Board of Immunology Letters

Member of the Editorial Board of Thymus

Member of the Editorial Advisory Board of Current Titles and Abstracts in Immunology, Transplantation and Allergy (CTAITA)

Associate Editor Bone Marrow Transplantation

European Editor of Human Immunology (from 1/9/1982)

ESHI Member Advisory Board European School of Hematology

Member of the "Stiftungsrat der Paul Ehrlich-Stiftung"

Member of "Scientific Advisory Committee of the Anthony Nolan Laboratories"

Member International Advisory Committee Netherlands Cancer Institute

Member of the Academia Europaea

AWARDS

1973	Recipient of the Dutch Kidney Foundation Prize
1977	Recipient of the Karl Landsteiner Memorial Award
1977	Recipient of the German "Spende eine Niere" Prize
1977	Recipient of the Robert Koch Medaille
1978	Recipient of the Wolf Prize
1980	Price of the Dutch Federation of Medical Research Societies
1985	Recipient of the Artois-Baillet Latour Price for the Benefit of Health, Brussels
1989	Recipient of the E. Jung-Prize for Medicine, Hamburg
1990	Recipient of the James Blundell Award of the British Blood Transfusion Society, London
1990	Recipient of the Amsterdam Prize for Medicine of the Royal Dutch Academy of Sciences

HONORARY LECTURES

1967	John G. Gibson II Lecture for Columbia University
1983	Heberden Oration for the Heberden Society, London
1984	Recipient of the Franz-Oehlecker-Medaille of the Deutsche Gesellschaft für Bluttransfusion and Immunhamatologie e.v.
1990	Niels Jerne Lecture, Les Embiez
1990	P. Grabar Lecture, Paris

HONOURS

1968	Doctorate Honoris Causa of the Faculty of Medicine of the University of Santiago de Chile, Chile
1970	Distinguished Service Decoration of the Dutch Red Cross
1972	Korrespondierend Mitglied Deutsche Gesellschaft für Bluttransfusion
1979	Honorary Member of the American Association of Immunologists
1980	Honorary Member of the Scandinavian Society of Immunology
1980	Doctorate Honoris Causa of the Faculty of Medicine of the University of L'Aquila, Rome
1980	Doctorate Honoris Causa of the Faculty of Medicine & Surgery of the University of Torino
1981	Doctorate Honoris Causa of the University of Maastricht, the Netherlands
1982	Doctorate Honoris Causa of the Faculty of Medicine of l'Université Claude Bernard de Lyon, France
1984	Corresponding Member of the Royal Academy for Medicine of Belgium, Brussel
1984	Doctorate Honoris Causa of the Faculty of Medicine of the "Katholieke Universiteit" of Louvain
1985	Doctorate Honoris Causa of the Faculty of Medicine of the Medicine of the "Université Catholique de Louvain", Louvain
1985	Recipient of the Order of Knighthood of the Dutch Lion
1988	Doctorate Honoris Causa of the Faculty of Medicine of the University of Essen
1989	Fellow of the Royal College of Physicians, London

RESEARCH ACTIVITIES have centered around the following topics:

1. Genetics of histocompatibility antigens
2. Organ and bone marrow transplantation
3. Genetic factors in disease
4. Bloodbanking
5. Clinical haematology
6. Clinical immunology

SYNOPSIS OF RESEARCH ACTIVITIES

In the last decade, a multitude of studies has shown that the HLA system plays an important role in the efficacy of blood component therapy, the prognosis of organ grafts and in the susceptibility for or the resistance against a great number of infectious and non-infectious diseases.

These studies were all dependent on the possibility to recognize the HLA system. The recognition of the HLA system only became possible in the early sixties. Before that time several workers (Miescher, Dausset, Payne, Van Loghem, Lalezari, Van Rood) had shown that antibodies against leucocyte alloantigens could be induced by blood transfusion and/or pregnancy. Although Dausset (the antigen Mac), Miescher and Lalezari presented formal proof that the antibodies were alloimmune antibodies and recognized white cell antigens, the leucocyte antibodies proved difficult to work with and a systematic approach to classify leucocyte antigens was lacking. This changed in 1962 when Van Rood published such an approach.

In brief, the sera from a large number of women who had formed leucocyte alloantibodies during pregnancy were tested against the leucocytes obtained from 100 unrelated donors. A computer-assisted search identified two clusters of sera with high internal consistency i.e. they reacted or did not react with the leucocytes of approximately the same donors. The determinants so recognized were of contrasting specificity and could be shown to be coded for by allelomorphic genes. They are now known as HLA-Bw4 and -Bw6.

The importance of this discovery did not lie only in the fact that it was a first description of diallelic antigens in the HLA system but also and above all in providing a systematic approach by which the complexity of the HLA system could be unravelled. Using this approach, Van Rood and collaborators described a total of 9 HLA antigens and a diallelic system not linked to HLA (the group Five system). Using the same approach, Payne and Bodmer detected three additional antigens. Together with Balner, Van Rood demonstrated that these leucocyte antigens were in fact transplantation antigens. In 1965, Dausset et al. again using the same approach, described several additional antigens. At the same time but independently, Dausset and Van Rood suggested that the majority of these antigens might belong to one system; Dausset on the basis of association data, Van Rood on family data.

An intensive international cooperation made it possible to unravel the complexity of the HLA system further.

During these studies it became evident through observations by Amos, Bain, Eijsvoogel, Yunis and notably Bach, that the HLA system coded for determinants which were able to induce blast formation in allogeneic T lymphocytes. These determinants are coded for by the HLA-D locus and can be studied in the MLC test originally described by Bach, et al and Bain, et al.

In 1973 Van Rood and his collaborators described a serological method to identify antibodies which reacted with these HLA-D determinants or closely related structures. They are referred to as the HLA-DR determinants. Many of the genes which predispose for the diseases mentioned above, are identical to or closely associated with these HLA-DR genes.

During the studies on the genetic complexity of the HLA system, a conscientious effort was made to implement the information so obtained in clinical and preventive medicine, as can be exemplified as follows:

1. The first HLA matched platelet transfusions in the treatment of aplastic anaemia were given in Leiden in 1964.
2. After demonstrating in a retrospective study that HLA matching could improve kidney graft survival, the Eurotransplant organization was established in 1967 by Van Rood. Eurotransplant is an international organ-exchange organization which operated as follows: the leucocyte groups and other pertinent clinical and administrative information from patients awaiting a renal transplantation are stored in a central computer databank. Whenever a postmortal donor becomes available anywhere within the Eurotransplant area, a matching program indicates the location of the most suitable (HLA compatible)

recipients. Following example of Eurotransplant, national (Francetransplant and UK-transplant) and supernational organ-exchange organizations (Scandiatransplant) have been established. Eurotransplant maintains close contact with those organizations.

The results obtained in the Eurotransplant area are among the best in the world and enable many patients who otherwise would have to be treated by an artificial kidney to lead an (almost) normal life. The activities in Eurotransplant have not only led to improved patient care but also provided new possibilities for (clinical) research.

These included a possible explanation for the pretransplant blood transfusion effect. This seems to be dependent on a sharing of a class II or HLA-DR antigen between bloodtransfusion donor and recipient. A follow-up of this study has shown that non-inherited maternal antigens might induce a form of (partial) tolerance of the child towards the mother. This has possibly important consequences for organ and bone marrow transplantation and the pathogenesis of autoimmune diseases.

3. The Leiden group has from the beginning played an active role in supporting the establishment of numerous tissue typing centers within and outside Europe. Some of the more important centers are found in London (St. Mary's Hospital), Belgium (3x), Germany (7x), Turin, Oslo, Zurich, South-Africa (3x), North America (3x), Montreal, South America (2x), Australia (3x), Singapore, New Delhi, Tokyo, Moskow, and Budapest.

4. The application of the HLA matching in bone marrow transplantation also started in Leiden. The first bone marrow transplants which were performed there were done at the time that importance of HLA matching in organ transplantation was not yet clear. However, soon afterwards findings in Leiden itself and those from other countries established without doubt that matching for HLA made all the difference.

   Perhaps the most clear cut data came from the studies in children suffering from immunodeficiency. Without exaggeration it can be stated that HLA matching revolutionized the treatment of children with immunodeficiency by bone marrow transplantation. The first successful cases were performed in Minneapolis (Good, Bach) and in Leiden (De Koning, Dooren, Van Rood and others). Thanks to these activities, survival of SCID patients who have an appropriate donor is well over 80%.

   However, many of the children suffering from immunodeficiency do not have an appropriate donor. Van Rood suggested already in 1974 (Transplant Proc. VI 4: 411, 1974) to come to organizations similar to that of Eurotransplant but now called Europdonor. The genetics, mathematics, and logistics of such an organization we worked out in detail.

   At the moment in Europe there are only about two hundred thousand donors who are suitable to be used for bone marrow transplantation, have been HLA typed and have given permission to be contacted if a patient in need of bone marrow cells of their HLA type is presented. A part of the effort of this organization is embedded in the European Bone Marrow Transplant Group, an initiative of Van Rood, Speck and others, in which teams working in bone marrow transplantation and leucemia, aplastic anemia, autologous marrow transplantation and transplantation immunology and immunodeficiency collaborate through multicenter protocols and meetings.

   Van Rood has been the Chairman of the latter group since the start of the European Bone Marrow Transplant Group. One of the goals of the organization is to actively support and stimulate the use of unrelated donors in bone marrow transplantation for patients suffering from immunodeficiency. To this end Van Rood persuaded the 10 different bone marrow donor organizations to pool the information on these donors in one single publication: "Bone Marrow Donors Worldwide", which has been widely distributed in Europe and the USA.

5. HLA and disease.

   The Leiden group has from the beginning been involved in the study of the relationship of HLA and disease, such as leucemia, Morbus Hodgkin, gluten enteropathy, ankylosing spondylitis and others. A concerted effort has been made in the last years to study the genetic factors involved in the occurrence of major endemic diseases. A model for such studies was leprosy. About 12 million people are suffering from this disease in which failure of cellular immunity plays a major role. Systematic studies from the Leiden group have brought forward the following important points:

   a. The genetic predisposition for the disease is probably not governed by the HLA system but by a locus or loci outside the HLA system.
   b. The HLA system does play a role, however, in determining in patients who have been infected whether they get the lepromatous or the tuberculoid form of the disease. The tuberculoid form of the disease is far more frequent in patients who are carrying the DR3 antigen.

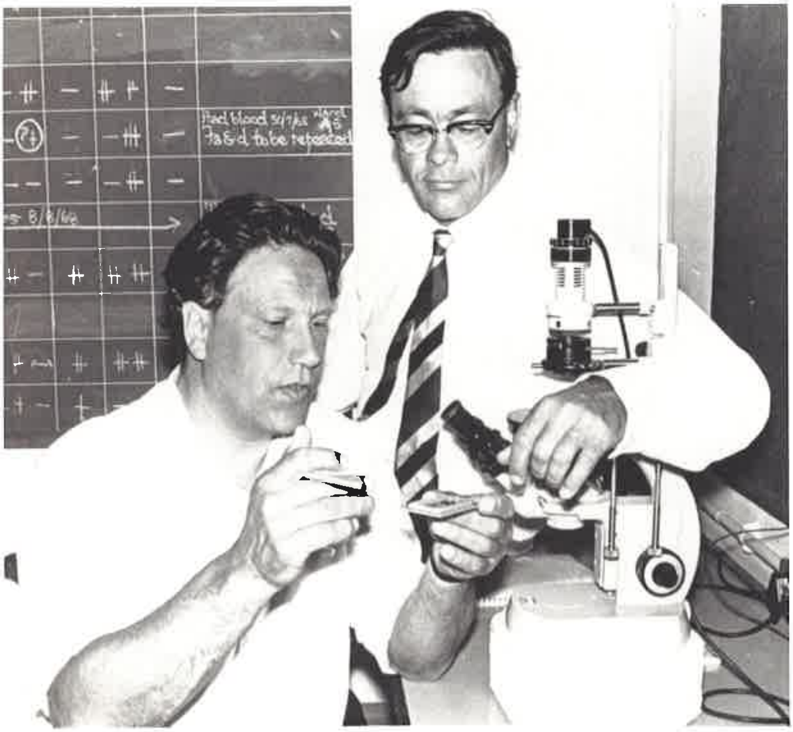
Leiden
1965

Felix Milgrom, WHO organ Exchange 1968

1961

with
Aad van Leewen
1967

1970



Jon
van Rood
with
Martin
Botha

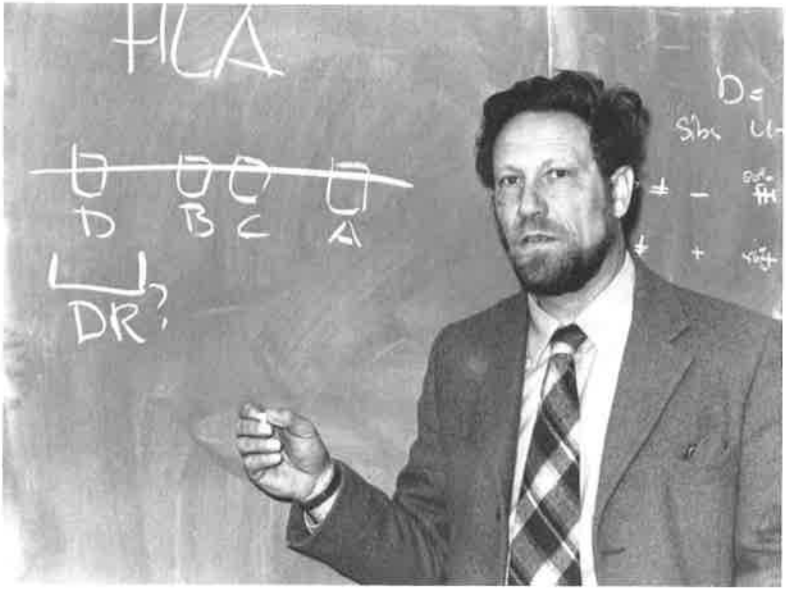
Capetown
So. Africa

Favorite place 1975

1982

Rudolf Pichlmayr

1975



1975

Jean Dausset *Jon van Rood* *Bernard Amos*

Marseilles, 1982

When
not
contemplating
on 4a 4b.......

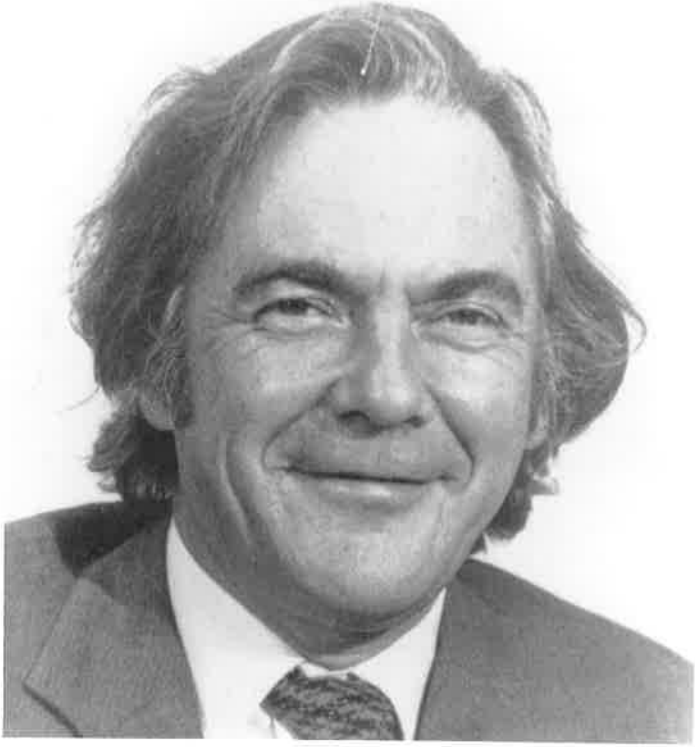
HLA — A Mouser's Recollections

D. Bernard Amos

Address: Duke University Medical Center
Division of Immunology
Durham, North Carolina 27710

D. Bernard Amos

1985



ca. 1960

ca. 1979

HLA — A MOUSER'S RECOLLECTIONS

D. BERNARD AMOS

There are few opportunities to open up a new field. In some senses to do so is exhilarating, in others it is frustrating and almost humiliating. When a new procedure works for the first time, or when you suddenly understand a reaction or produce a completely new reagent, it's exciting. When you just can't understand the relationship between cause and effect or when the only approach to a problem seems to be a slogging push-on type of approach instead of an inspired, intellectual solution—it's frustrating. We had plenty of each in developing HLA. Some things went so quickly and easily, like selecting our first HLA identical sibling kidney donor and then watching the patient bounce back to health—that's exhilaration. Trying to sort out cross-reactive antigens with a limited number of unknown antisera or trying to equate HLA type with function—that's frustration.

Looking back, in response to Paul's suggestion that we write a book, has made me remember how deeply involved we became in our problems and with each other. We developed a respect and affection for our colleagues and rivals struggling with the same problems, to an extent which is difficult for today's young scientists to imagine. Yes, we *did* have fun. We certainly worked hard; sometimes we strutted; but sometimes we were much too diffident! So, thanks, Paul, for giving us the opportunity to think of those times, 30 years ago, the times of the origins of HLA.

A few words often change the whole course of a life. Several times my own life has followed a completely new path as the result of a whim, a casual question or comment from a friend or acquaintance. The first turning point came when I skipped school, got on my bicycle and rode the three miles to Burroughs Wellcome Physiological Research Lab. I'd been keen on chemistry in secondary school. The war was interrupting schooling, and I felt it would be desirable to see a little of life before being called into the Army. Burroughs Wellcome were well-known in England as manufacturing chemists, so I'd thought to get a job in pharmacology or chemistry. Instead of a chemistry lab, I found myself in a microbiology lab and instead of a chemist as a boss, I had R. Gwynn MacFarlane (Mac), one of the most brilliant hemophilia specialists in England. He'd have been even better known if he'd been a little less brilliant and much less of a dilettante. A little of his brilliance and most of his bad habits were eagerly absorbed by his new helper. Mac wasn't suited to industry and soon accepted the position of Reader in Hematology at Oxford. I surprised myself by asking if I could go with him. He surprised me too by saying, "Yes." The next turning point came when a friend's father said, "you were always interested in medicine, why don't you become a doctor?" at a time when I was becoming dissatisfied with my life as a laboratory technician, too many new people were coming into the lab as the war ended. Since they all had degrees, I was going backwards. Another came when I was an intern looking for a start on a career in medicine. The Professor of Bacteriology asked, "why don't you talk to Gorer" after he had already agreed to take me on as a Research Fellow in his department. Following the question as to why I didn't go into medicine, I did, almost miraculously, manage to get into Medical School—Guys. Following the question about Peter Gorer I did, very hesitantly, go to see him to explain my ideas for immunizing against cancer. Instead of agreeably endorsing my proposal to make antiserum in rabbits as the bacteriologist, Knox, had done—Gorer said, "Nonsense." So of course, I ended up working for him instead of Knox. The medical degree gave me the freedom to spend the rest of my life doing what I wanted to do. Working with Gorer exposed me to one of the finest minds in medical research; but this is by way of explaining how I got into immunogenetics instead of working in an office or in a lab as a technician.

Another course change began on the shore of Lake Ontario. On a cloudless day in early summer, Fred Stone, Delta Uphoff, June Wakefield and I were watching sailboats out on the lake. Fred and Delta had been graduate students at the University of Rochester. Delta had gone into radiation biology and worked on the prevention of graft versus host disease. Fred had turned to science administration and was Assistant Director of the National Institute of General Medical Sciences (G.M.S.) while June was working with me on the destruction of transplanted mouse tumors. The work was exciting and the speed and intensity of the rejection mechanisms were awesome. Delta and I had earlier probed into graft versus host disease. We also shared interests in the interplay between genetics and immunity. We were of course animated by Delta's use of Methotrexate as an immunosuppressive agent in bone marrow transplantation and by the prospects of using drugs in organ transplantation. Fred had been content to listen and to keep the conversation lively by asking questions from time to time. He suddenly changed the direction of our discussion by talking about the ways in which administrators could work with scientists. Delta and June and I had become animated in our discussion, then it was Fred's turn as he showed us that perceptive administration could be just as exciting and just as rewarding.

In the 1950s great advances had been made in coaxing premature infants through the first weeks of life. More and more frequently premature babies were being kept alive by a variety of new procedures. There was an unexpected price for this. Many of the infants were later found to be blind. The G. M. S. institute had joined forces with pediatricians, biochemists and physicologists and in a brilliant (and well funded) collaboration had shown that retinal degeneration was an unexpected consequence of excessive oxygen tension. Fred proposed that it would be possible to harness our knowledge of the genetics of tissue rejection in the mouse and of drug induced immunosuppression in animals to make organ transplantation practicable in man.

This prospect excited me because Hume, Murray and their colleagues had recently reported on their limited but revolutionary success in transplanting kidneys from human cadavers to patients with end stage renal disease. Together we explored Fred's suggestion that the time was right for a serious attempt at elucidating the genetic and immunological bases of human transplantation.

A few months later, Fred asked me to arrange a dinner during one of the New York Academy of Science's Tissue Transplantation conferences. At this informal meeting (Rupert Billingham assures me it was financed by a drug house although I did not know this at the time), sixteen or so clinical and laboratory experimentalists sat around the table after dinner and debated the proposition that the genetic basis for human organ transplantation could be unraveled using the background knowledge acquired from inbred strains of rats and mice. Somewhat to my surprise and much to my relief, there was no serious dissent by the clinicians led by Joe Murray, Georges Mathe', David Hume, and John Merrill. Many useful suggestions were made as to how best to approach the basic problems.

Afterwards, Fred said he thought the General Medical Sciences Institute might be willing to establish two institutes for transplantation research, one in Europe and one in the U. S.

As a first step towards this, Fred Stone and I made a brief pilgrimage to some of the European Immunology and Immunogenetics laboratories. The object of the journey was to learn what was already being done or was proposed and to see which European center offered most opportunities for the proposed institute. Two of the most dramatic incidents occurred in The Netherlands and in Belgium. In Amsterdam we were introduced to a young hematologist, previously unknown to us. The meeting took place over lunch in a large underground beer hall. The young hematologist, who later became a life-long friend, was Jon van Rood. Jon was generous in sharing his thoughts as well as his data, and we had a fascinating conversation–so much so that I haven't the slightest recollection of what we ate. I remember very clearly, however, the graphic representation of the 4a, 4b (Bw4, Bw6) system later published in his thesis (1). Jon also said that he had recorded many other reactions which were not included in the diallelic system he had observed. This excited me because it accorded well with my own feelings about histocompatibility antigens in the mouse.

I now need to digress into H-2 because our (erroneous) misinterpretation of the H-2 system influenced the interpretation of the human data by others as well as myself. The serology of H-2 was almost exclusively worked out by Peter Gorer, first as a xenoantigen detected with his own (group A) serum and rabbit anti-mouse serum (2) and later by alloimmune sera (3). When I joined Peter in 1951, he was trying to overcome some of the technical difficulties inherent in mouse red cell serology. Four mouse strains were available—A, Balb, CBA and C57BL. Gorer could detect an agglutinin in the serum of C57BL mice immunized against Balb or A tissues by testing against CBA or A cells; reactions of antisera raised in A against the EL4 lymphoma of C57BL were very difficult to detect. In general A cells were very easy to agglutinate, CBA were somewhat less easily agglutinated, Balb cells gave variable reactions and C57BL cells were usually inagglutinable. At this time, in the early 1950's, there was much discussion about incomplete antibodies, a spill-over from Rh serology. Gorer believed there were "no incomplete antibodies, only inconvenient antibodies." He needed a technical breakthrough before he could get much further with the planned analysis of normal tissue antigens.

A central problem was that H-2 behaved in many respects as a single antigen with several alleles; but in other respects it was more like a complex antigen reminiscent of Rh. Gorer thought there were two closely linked loci. Snell did not share this viewpoint, so Gorer felt that the only conclusive proof would come from a recombinant that would separate the two or more closely linked genes. Detection of recombination (or crossing over) was impracticable while many of the antigens remained hypothetical and while the procedures gave erratic results. Similar problems beset HLA research ten years later. However, Gorer noticed that the test became more sensitive in the winter. He was able to use this chance observation to make a tremendous advance in mouse serology. Mouse cells, being fragile, are usually suspended in serum. Gorer usually used his own, absorbed, serum. During the winter serum was often collected while he was recovering from one of his frequent throat infections. Reasoning that convalescent serum was enriched with immunoglobulin he tried other colloid rich fluids including myeloma serum, pseudomucinous cystadenoma fluid and finally, taking a tip from Grubb, dextran. These investigations which were very time consuming were in progress when I joined him. He felt he needed a backup in case of failure; he asked me to work out another assay. His first suggestion was for a lytic assay using cultured mouse embryo explants as targets. This investigation ran into a whole series of snags not the least of which was caused by the yeast which blew in the open window from a nearby hop warehouse. Another block was due to the inefficiency of mouse complement in lysing mouse cells. Although both of these were eventually overcome, the first by adding dilute hypochlorite to the cultures and the second by substituting rat complement, progress was slow and he suggested I try to work out a leukocyte agglutination test instead. After many unsuccessful attempts with peripheral blood, I harvested peritoneal exudate cells from animals injected with killed *M Tuberculosis* or BCG. To our relief the peritoneal exudate cells could be made into a homogeneous suspension that could be agglutinated by alloantibody. Furthermore, the agglutination patterns were all we had hoped for. Unlike red cells from C57Bl mice, their leukocytes were just as agglutinable as those from other strains (4). By the time leucoagglutination had been developed, the various techniques Gorer and his technician, Barbara Mikulska had devised for red cells were working well. We now had the two different approaches for detecting "inconvenient" antibodies and could combine forces looking for an intra H-2 recombinant. Gorer and Mikulska set up one set of segregating crosses, and I set up another. The Gorer-Mikulska segregating crosses could be tested directly with antisera against D (H-2.4) and K (H-2.11) and mine could only be tested through in vivo absorption—another procedure that had been worked out in the course of some antitumor experiments. Both of our attempts to detect crossing over were successful (5,6). I had only 32 back-cross mice so was extremely lucky in finding a recombinant in these few segregants. Gorer and Mikulska had more animal space and could breed more mice. They had 2 recombinants (D+K– and D–K+) out of about 250 segregants tested (6). The antigenic markers detected in my cross were E (H-2.5) and B (H-2.2). Having the 3 different recombinants D+K–, D–K+ and E-B, we could re-analyse our antisera and could define many new antigens. Gustovo Hoecker and his wife, Olga Pizarro, fellows in Peter's laboratory, and Jack Stimpfling at Bar Harbor later tested other strains of mice. Between us we soon described numerous recombinants and had detected more than 26 antigens so had to change to a numerical terminology. Because each new recombinant found seemed to make another split, we regarded the genes as being like beads on a string. Although this misconception was almost universally accepted and an accepted dogma is all pervasive, there were always some difficulties. For example, attempts to separate D (H-2.4) from F (H-2.6) were unsuccessful even though over 400 segregants were tested. There was also abundant evidence that many of the antigens were highly cross-reactive, a possibility predicted by Ray Owen. This was shown with anti-K (H-2.11) which was completely absorbed with Q (H-2.16) just as anti-HLA-3 was later completely absorbed with cells carrying HLA-11. An inconclusive search was made for diallelic pairs within H-2; C (H-2.3) and V (H-2.22) appeared to constitute a probable pair of alleles for example. For some reason Gorer resisted this supposition. However, the terms "public" and "private" antigens were introduced and proved very helpful even before the cross-reactive clusters governed by H2D and H-2K were recognized by Klein and Schreffler (7).

Thus, when van Rood told Fred Stone and myself of the di- allelic pair 4a and 4b, the human alleles appeared to me to be comparable to H-2.3 and H-2.22. The additional reactions observed by van Rood would be like the private antigens of the mouse. This confirmed my prejudice that human antigens would follow the mouse model. One idiosyncrasy of the mouse antigens, unrelated to H-2, proved not to occur in man. Besides the numerous H-2 antigens, my colleagues and I had detected a number of antigens which were not part of the H-2 system. For these we used Greek letters, alpha, beta, etc., later H-5 and H-6 (8). These antigens were erroneously labeled Ea5 and Ea6 by a nomenclature committee since they were present on red cells. Ea was a serious misnomer since both H-5 and H-6 had a widespread and well documented tissue distribution. We should now call them differentiation alloantigens. These and other "non-H-2" antigens deserve further investigation with current techniques and insights especially since one of them, Theta, has achieved great distinction. Discovered and named by Reif using the approach we had used for other antigens, Theta, later renamed Thy 1 with alleles Thy 1.1 and 1.2, is also a differentiation alloantigen (9).

For me, the meeting with van Rood was most exciting and decisive. Human alloantigens, like those of the mouse, were amerable to attack! Another meeting during our visit to European laboratories was very exciting and left an equally indelible impression. Unfortunately, little concrete has come from it. George Lejeurne and Andre' Castermans were working on novel methods of preparing soluble antigen. When we visited their laboratory, Lejeurne also told me of some experiments on neonatal tolerance performed on children who had received exchange transfusions, as newborns, for hemolytic disease. He was particularly excited about a graft from an older male donor to a young girl. The graft had persisted for several years and had actually grown as the child grew. He took me several miles to the child's house and showed me a graft about 5 cm in diameter, smooth, supple and with no induration or scarring. This graft had all the stigmata of skin from an older person. There are two such series of grafts to "tolerant" children in the literature, that of Lejeurne and another by West et al. from Cleveland (10,11). In each a proportion of grafts lasted for months to years. Lejeurne was later severely criticized when he reported that the skin donor was not the exchange transfusion donor but had donated blood for another infant transfused on the same day. It has been suggested that the "graft" was a scar, but I can't believe it and I have seen many grafts which have persisted for 4-8 weeks as well as many scars. The contrast between the tense, firm skin of the child and the darker pigmented, distinctively patterned and less elastic skin of the graft was unforgettable. Unfortunately, the parents considered the graft unsightly and would not permit any further manipulation, so no biopsy was performed. I still question the fine specificity of immunologic tolerance in man and regret that no further studies are possible.

On my return from the European laboratories, a report was prepared for the General Medical Sciences Council and presented to Phillip Handler, Chairman of the Council. Handler was also chairman of Biochemistry at Duke and an unofficial ambassador for the Dean, Barnes Woodhall. Handler was impressed; as a consequence I was shortly thereafter offered a position at Duke. Leaving most of the mice to the Buffalo climate, I switched to Duke and studies of human leukocyte antigens. The surgery department had a young surgeon, Del Stickel, who was sent to Edinburgh for training in transplantation and Del and I together with Jim Glen, urologist, Ike Robinson and Caullie Gunnels, nephrologists, set up the Duke renal transplantation program.

The first months at Duke were trying, working in a nearly bare laboratory, waiting for new space and trying to learn an entirely new methology. I had had one previous attempt at human leukocyte serology when Gorer's laboratory at Guys had to close because of an outbreak of mouse pox brought in by mice used for drug toxicity testing by the pharmacologists. One problem with human leukocyte agglutination in 1953 was that the buffy coat cells used were too agglutinable; the controls as well as the tests agglutinated. A colleague, Patrick Clarkson did, however, publish one brief paper on an attempt to detect antibodies to skin in a burned child. There was no incentive to continue once a healthy colony had been re-established, some of the animals which had escaped infection having been bred up in our bathroom at home! At that time mouse histocompatibility antigens were much easier to work with than human.

Dick Metzgar, who also came to Duke from Buffalo, worked on non-human primate leukocyte antigens and was later joined by Chester Zmijewski, an immunohematologist and another former Buffalonian. I was able to recruit two enthusiastic research technicians, Nancille Peacocke and Jean Nicks. Nan helped me with the leukocyte serology and Jean with some of the functional assays, these two being our main lines of research.

In serology, we had two problems that now seem trivial, but were at the time almost insurmountable. Anti-sera were difficult to obtain; we had no real experience with human leukocytes. The successful mouse leukoagglutination had been with peritoneal exudate cells, and although we did look at human acetic fluid it was poorly cellular and not a very promising prospect for any systematic study.

Most of the techniques then in use had been worked out by hematologists who were not interested in transplantation but in autoimmunity to white blood cells. Such pioneers as J. J. van Loghem, N. R. Schulman and T. E. Brittingham had worked on agglutination. Other hematologists had adapted complement consumption, antiglobulin consumption and platelet aggregation for their studies on autoimmunity (12). Most of the methods were extravagant of materials, insensitive and subjective. One of the biggest obstacles to standardization was the large volume of serum required and another was the short life span of the indicator cells. van Rood used two drops of serum, i.e., about 100 l, per determination and all determinations had to be done in duplicate—usually on more than one occasion. Payne also used 50 l per tube as did Dausset. Some tests required as much as 0.2 ml. Since plasmaphoresis was unknown and it was usually difficult to obtain more than 5 ml of blood from a donor, many of the sera were half consumed before they had been characterized. We were lucky in that we were able to transfer technology from earlier mouse studies, dispensing 20 or 25 l from Pasteur pipettes that has been graduated with mercury, using polyvinyl pyrrholidone (P.V.P) as a superior erythrocyte sedimenting agent and carrying out all tests in siliconized glassware (13).

My own entry into the human leukocyte antigen field, like that of Terasaki and Batchelor, was late. Rose Payne referred to us as the mousers. We soon found that most of the tests required suspensions of live leukocytes. Fixed cells and stored cells would not agglutinate. The anticoagulant influenced the reaction and so did the temperature of cell preparation and incubation. Limited serum exchanges occurred but were not always rewarding for the donor laboratory. Because methods differed from laboratory to laboratory, the shared sera did not always behave as expected and, regrettably, credit was not always given to the donor laboratory for help in identifying a new antigen. It was not easy for a new investigator to enter the field. Availability of serum was a tremendous problem. Until van Rood and Payne independently discovered that antibodies were to be found in pregnancy serum, the main sources were transfused patients. These were frequently very sick people so only very small volumes of blood could be drawn, repeat samples were often impossible to obtain and specificities were apt to change following blood transfusion. Even when it was known that parous and post-partum women might have an antibody the medical practices at the time usually forbade the collection of anything but small samples and in some countries even this was forbidden, leaving the diluted blood escaping from the vagina at the time of birth as a major and sometimes grossly contaminated source.

My own laboratory was fortunate in receiving a generous gift of 10 ml of an antiserum from Rose Payne and smaller quantities from van Rood. The Payne serum was multispecific and of no value as a typing reagent, but it was invaluable for working out an agglutination technique that worked well for us. We were also fortunate in having a close relationship with Roy Parker, a senior faculty member and later chairman of the obstetrics department, and Harry Honeycutt, a resident who became interested in our research. Harry assiduously collected blood from women who had a history of multiple abortions. Seventy-five percent of white women with obstetric difficulties, including placenta previa and recurrent spontaneous abortion, and a slightly smaller proportion of Blacks produced agglutinating antibody, compared to approximately 30% of multipara with no obstetric problems (13).

Like everyone in the field, we developed a method that suited us best. Platelets and excess red blood cells were a hindrance to easy reading of results. Different laboratories dealt with these in different ways, defibrination, hypotonic lysis of red cells, hemagglutination with anti-red-cell sera, the addition of phenol and sedimentation with polybrene were among the procedures used. These could all influence the result but none more than polybrene, which we tried. Fortunately for us, only some batches of polybrene were effective and ours was not so we switched to P.V.P. which had worked well with mouse cells. Lalazari, who developed the polybrene method, had samples that worked admirably. However, because the resultant suspensions were neutrophil rich, he discovered the NA series of Neutrophil Agglutinating sera rather than the HLA antibodies which react with lymphocytes. In my hands, neutrophils had little or no HLA antigenic activity. They were, however, essential for leukocyte agglutination. I don't believe that the reactions which we labeled "leukoagglutination" were a true agglutination but a form of aggregation which followed the interaction of antibody with mononuclear targets. Not infrequently red cells were also included with the mixed aggregate. There was a specificity to this phenomenon involving red cells which was not investigated. The presence of platelets interfered with tests in which the antibody had been tissue absorbed.

My own first contact with the other pioneers in the field was in 1963 at a Hematology Congress in Lisbon. This meeting was attended by the leaders in leukocyte serology who were predominantly hematologists. Walford was a pathologist and hematologist who had written a major monograph on white cell antibodies, but Payne, van Rood, Lalazari, and Dausset were primarily hematologists. The other newcomer, Ceppellini, was a geneticist. I was nervous and glad to get my first paper over. It didn't seem to have much impact although it was a very comprehensive paper including a preliminary report of absorption with a variety of cells and tissues. Perhaps it was too comprehensive, but at least it was not controversial. Lalazari, who was at the time engaged in a dispute with Rose Payne over the significance of Neonatal Neutropenia, did, I believe, get involved in a controversy as to the significance of this condition. He and I felt somewhat outside the mainstream and spent some time together including an interesting evening at a Portuguese bullfight. Ceppellini and I found we were both headed for the Genetics Symposium in Amsterdam and we both wanted to visit van Rood; we also found that neither of us had airplane reservations out of Lisbon. This was serious as all planes were fully booked. When we arrived at the airport, we found several hundred people were already struggling for places and the main concourse was jammed. Then I had my first experience of Ruggero Ceppellini's charisma and resourcefulness. He led me away from the crowd to the Administrative Offices where he asked for the Airport Manager. Told he was away until afternoon, Ruggero charmed his assistant into giving us a 2:00 p.m. appointment with the chief. After a leisurely lunch, we went back to the Manager's Office and were admitted. With a low bow and his hand over his heart, Ruggero introduced us—"I am Professor Ceppellini and this is Professor Amos, a famous geneticist from America." Once convinced that the future of genetics lay in our getting to The Netherlands that same afternoon, the manager led us to the transit lounge. A half hour later we, together with the Brazilian National Soccer Team, boarded a Varig airliner and, sole passengers, traveled first class to Amsterdam. After some inquiries, Ruggero and I boarded an airport bus headed for Rotterdam and descended near Leiden where we took a taxi. All the best hotels were full, the taxi driver then made the rounds of the second-class hotels, all full. Finally, the resourceful driver said, "I do know of a place you can stay," and took us to a restaurant with rooms which we believed were usually rented by the hour, above the dining room. We had an interesting evening and arrived refreshed next morning at the laboratory where Aad van Leeuven kindly brought out the lab notebooks containing the individual reactions of 4a and 4b. Ruggero was interested in the apparent excess of heterozygotes and, as always, a skeptic, he wanted to check the raw data for Hardy Weinberg equilibrium. We were both impressed, not only by the data but by the generosity of van Rood and of van Leeuven who allowed us free access to it in van Rood's absence. The apparent discrepancy was later shown to be due to ANAP, agglutimation negative absorption positive cells in the test panel.

I must now make a second digression to discuss the genesis of the Workshop meetings that became so important to the development of HLA.

Allusion has been made to the Tissue Transplantation Conferences of the New York Academy of Sciences. These meetings, organized by John Converse and Blair Rodgers, were the most prominent of the meetings that brought immunogeneticists together. The New York Academy Series later spawned the Transplantation Society. These were open meetings that later got very large. There were also a number of closed but very important meetings. In England there had been the Ciba Symposia and I remember most vividly a presentation given by a very young man with four initials. This was of course JFAP Miller and his topic was the function of the thymus. This was a breath-taking novelty as before his work, the thymus was an anatomic oddity with no known function. Other small gatherings held in the late '50's and early '60's also had a great impact. One was the Liege Symposium organized by Albert and Medawar and another, the Ford Symposium. Most of the smaller meetings were sporadic. Included in their number—but with greater continuity—were the meetings organized by the Committee on Tissue Transplantation of the National Research Council (NRC)—National Academy of Sciences, chaired in a most unusual manner for a number of years by Ernst J. Eichwald (Eich).

Eich's committee met at irregular intervals. There appeared to be no particular term of service and no fixed number of members, certainly there was no large budget to work with. Eich would call a meeting and we would discuss recent developments. Sometimes we would adjourn with little obvious having been accomplished, sometimes we would meet to plan a scientific meeting, at which we would invite several experts to come and present their ideas and their data. These meetings, which were very stimulating, would be summarized as a report to the NRC. Papers from some of the meetings were published. One, on Cell Bound Antibodies, edited by Hilary Koprowski and myself, set records for speedy publication. I did most of the editing while Hilary did all of the work involving publishing which was by the Wistar Institute Press. Since Hilary was director, everything flowed smoothly and rapidly; publication was complete in less than 2 months. Another such publication was on the thymus.

One of the most momentous meetings of the Tissue Transplantation Committee, however, was organized in late 1963 and took place in June 1964. This was the conference on Histocompatibility Testing, chaired by Paul Russell and followed immediately by the First Histocompatibility Testing Workshop. I'm usually given all the credit for this meeting, understandable if one individual is to be singled out, but in fact, the suggestion for holding a bench workshop was brought forward in the committee; it's possible it was first voiced by John Trentin. At any event, the whole committee enthusiastically endorsed it because such a workshop was urgently needed because the field was so fragmented by individuality in methodology (14).

The conference part of the meeting was very successful, the social side was also quite pleasant and a number of us sampled the entertainment on K Street for relaxation; one of our number enjoyed the belly dancers so much he was nearly ejected from the bistro. Then, having completed the traditional part of the meeting, a selected few went down to Durham for the untried medium of a laboratory workshop.

The Workshop was a "state of the art" meeting denoted to functional tests as well as to serology. Among the functional tests the Mixed Leukocyte Culture Reaction (MLC or MLR), demonstrated by Kurt Hirschhorn and by Louis Lowenstein and Barbara Bain, and the Normal Lymphocyte Transfer Test presented by J. G. (Ian) Grey each seemed to have enormous potential. These were both quite new. The other major functional test, skin grafting, had been adequately described at the preceding New York Academy of Sciences meetings. A novel approach presented at the Washington meeting, was to couple skin grafting to adoptive immunity conferred on an unsensitized donor by transfer factor. This was and still is, a tantalizing procedure. Then, at the First Workshop, Felix Rapaport demonstrated his simplified procedure which had many advantages over those used by plastic surgeons.

Chester Zmijewski, Bill Boyle and I together with my graduate student, Brack Hattler, and our technicians had been working for several weeks to prepare our laboratories. We had two main areas, 625 square feet of laboratory space on the second floor and 350 square feet of laboratory space on the third floor of adjacent buildings, the use of a small lecture hall, and two lab-offices which we could use for informal discussions. The larger laboratory was set up for serology and the smaller for functional tests. Each participant had sent us a list of equipment and supplies several weeks earlier; we hoped that everything would be ready by the time the participants arrived from Washington. Each of the serologists had been asked to bring enough antiserum to share with the others. The main objective of the Workshop was to compare procedures using the same serum and cells. Bill, Chet and I had decided that we would not use our own sera because these were unknown to the other investigators and in all probability would not be accepted. There was no question about the cells. These had to come from our panel since cells could not then be shipped.

The Workshop began with a reception at the Holiday Inn. North Carolina was a dry state but alcohol could be bought at State Liquor Stores and could be served at private parties, so we had hired a hall and provided suitable refreshments. Chester and I went around asking for serum to set up for the morning. To our dismay no sera were forthcoming and for a while, it seemed as if we would be limited to mock displays whereas what we had hoped for was to have each of the senior serologist give a demonstration of his or her procedure using the best possible shared reagents. Ceppellini suggested we might just have enough donated serum to make up sets, so Chester and I slipped away and dashed back to the lab where we checked the freezers. We did indeed have about 1 ml each of six sera, one each from the major laboratories, so we could smile as we rejoined the party which was now going very well. In the morning we picked up the participants and showed them to their benches where six blood samples and the six sera had been distributed. Much to our relief all but one of the visitors began to prepare cells. Those setting up the functional tests also began their own preparations (15).

Within an hour or so, it was clear to us that the Workshop would be a success whatever the outcome. Making cells and setting up were time-consuming so there were intervals of free time while cells were sedimenting or being centrifuged. During these quiet periods, our guests could move around to see what their neighbors were doing. As soon as we heard comments such as, "Oh, is that the way you do it, it doesn't mention that maneuver in your papers," we knew that mutual respect and communication were being established.

Although my own focus was on agglutination, we were conscious of its many inadequacies. My own experience in the mouse had shown the advantages of a cytotoxicity test but attempts to transfer the procedures which were successful in the mouse, using guinea pig complement, to man were not effective. We had observed lytic reactions with some sources of complement including dog and sheep but backgrounds were unacceptably high or the reactions were only partially complete. Walford and also Terasaki had successfully introduced rabbit serum as a complement source and Terasaki participated at the Workshop. Not only did he demonstrate lytic reactions, he also embarked on attempts to miniaturize the test. The importance of this was apparent to everyone and Paul received not only lots of encouragement, he also received many suggestions. I don't know if any of them were useful! Other serological techniques were also compared including antiglobulin consumption, leukocyte and platelet complement fixation, and mixed agglutination (mixed hemabsorption). In some respects it was a tower of babel because so many different things were going on at the same time, but it was also a wonderful catharsis. As mentioned earlier, we had set aside two small rooms for informal discussion. I joined in some of these and would have tape-recorded these informal sessions but feared that the spontaneity would have disappeared as soon as a microphone appeared.

Ceppellini appointed himself scorekeeper and statistician. We wanted to make as many comparisons as possible, between the EDTA and definition methods for example, or between agglutination, cytotoxicity and complement fixation and so on. In addition we wanted to learn something of the sensitivity and practicality of the procedures. At the end of the day, Ruggero duly went to each bench and copied out the results. He noticed that one group was preparing to work extremely late. Ceppellini thought this peculiar so went back to the lab himself and found the late worker surreptitiously copying other investigators' results. Ceppellini had a vigorous and expressive flow of language; that investigator took no further part in the Workshop. The rest of the group went over to the Zmijewski's house where Helen provided a most elegant and relaxed dinner. The following day was a repeat of the first, but for the Duke group, without the tension because, to us, the Workshop was already a success. By now both laboratories were in full swing with the functional tests being read and compared while a new set of cells was being tested by the serologists. To me the Workshop seemed as active as a three-ring circus, one in which I was also a performer. We couldn't take functional tests like skin grafts or mixed leukocyte cultures to their conclusion, but we could approximate these tests. Felix Rapaport, for example, could exhibit skin that had been grafted two days earlier and demonstrate how to visualize the capillaries through a surgical microscope. Ian Gray could demonstrate the inflammatory response to transferred lymphocytes transferred on day one and read on days two and three, and so on. Barbara Bain brought previously established cultures for examination under the microscope. Felix Milgrom and Dick Metzgar each had a variant of the hemabsorption test using red cells as indicators or antigens on cultured cells, through an antiglobulin bridge. This was a very sensitive test but not sufficiently flexible for either antibody screening or donor selection and this exquisitely sensitive procedure has been dropped. Incidentally, I later found that it could readily be adapted to lymphocyte suspensions since they will rosette red cells after antibody treatment. Mixed agglutination could easily be used for exploring minor H antigens but it has now been superseded by other binding assays.

The evening dinner was at my home in the country. Recollections include Leslie Brent sitting tall on an even taller horse and Michael Schlesinger finding his horse would only proceed in one direction, back to the barn. My wife fed the party outdoors on broiled Cornish game hen, then quite a novelty. I think these social gatherings had a great influence on the interpersonal relationships and great camaraderie that developed in future years. This empathy also helped the discipline to advance rapidly.

On the final day, a Saturday, we had a number of presentations and more formal discussions which were recorded; I still possess the tapes. The Workshop concluded with Ceppellini's presentation of the results. Getting up on the stage, he produced a crumpled piece of paper and started to copy some scores onto the chalkboard. He apologized to the audience because the presentation was incomplete and explained that somehow the real summaries had been thrown out. These fragments were all that survived. Of course we were all disappointed but we were also very happy that two of the laboratories (mine was not one and neither was his) had almost completely concordant results. Only later did he confide that these were the only results in agreement, the others had been so bad he thought it best to destroy them!

In retrospect the results did not matter. What did matter was the rapport that had been established between the 23 investigators attending the Workshop together with a general acceptance of the sharing. We all learned a great deal from the discussions and informal exchanges. We all gained immeasurably from the inclusion of different types of procedures, functional as well as serologic. We also learned that cytotoxicity was a practicable procedure giving very different results from agglutination—in fact, as later shown by Payne, Bodmer and Terasaki (16), and then by Chester and myself (17), the two procedures often detect different antibodies in the same serum. During the course of the Workshop it became apparent that the meeting was approaching two very different objectives. This has reoccurred during subsequent workshops and the two objectives are sometimes difficult to reconcile. One objective is to recognize and agree on a new of antigenic specificities and the other is to help new laboratories attain a greater degree of proficiency.

Would we have been much further ahead if the Workshop had been more conclusive? I doubt it. The Workshop gave us all the impetus we could handle. It made us all much more friendly and cooperative. It also made us much more competitive and respectful of our competitors.

Some of the conclusions reached at the First Workshop were that we needed to develop new micro or semi-micro procedures that had both good sensitivity and freedom from the errors of false positive or false negative reactions. It was agreed that future testing should include two or more procedures run in parallel and that a number of sera with the same apparent specificity should be used. It was further agreed that antigenic differences must be correlated with graft rejection in some manner, but how this should be done was unclear. The Workshop proposed the setting up of two types of cell panel. An international panel would be the reference standard for testing new sera while secondary panels should be established on a more local basis. Since cells could not be stored or shipped, the investigators themselves constituted the walking international panel. One became accustomed to being accosted by a technician with a large syringe on walking into a strange laboratory following the cry, "He's one of the International Panel."

The need for continuing collaboration was stressed by Ceppellini in closing the meeting. How well that was heeded became apparent at the workshops that followed, especially during the 1965 and 1967 workshops which were still sufficiently small that the investigators themselves could work at the bench and the 1970 and 1972 workshops which although larger were still very closely knit. As we shall see later, the character of the workshops gradually changed and they have now become so enormous as to be almost unmanageable. It is sad that so much data has been generated in recent years that only small subsets have been analysed, much detail has been missed by the central analysis which has to restrict itself to an analysis which is often superficial.

After the visitors had left Durham, the lab staff had the less pleasant chore of putting the laboratories back together again. I was informed that if I ever volunteered Duke as host for an international workshop again, the faculty and staff would resign en bloc. This promise has not been tested because although we have organized many regional and training workshops, we have never hosted another international workshop!

Separate from the Workshop but later becoming intertwined with it was an initiative set-up by the National Institute of Allergy and Infectious Diseases (NIAID), a component of the National Institutes of Health. An ad hoc committee chaired by Rupert Billingham and Frank Dixon debated the needs of transplantation biology and formulated a list of objectives. One of the Branch Chiefs of NIAID, John Overman, formerly a microbiologist at Duke, was subsequently asked to set up a collaborative research program and invited me to serve as chairman of the review committee. I then asked Al Stetson from New York University to serve as vice chairman and we recruited the first committee on Transplantation and Immunity. Congress appropriated $200,000 for the first year's operation. The money was to be spent for the support of contract research. We decided that the most effective way to use these funds was to provide "add on" incentive money to ongoing programs. Top priority was given and to establishing a serum bank known as the NIH Serum Bank and to establishing contracts that would increase the flow of serum. The committee procured serum by awarding about $30,000 to investigators with an established program and who agreed to collect serum in excess of their own needs to be sent to the Bank. Serum could also be purchased directly by the Bank for, as I recall, $2.50/ml.

Sadly, John Overman died suddenly during the early days of the Committee's operation. This was a severe blow to the program, but fortunately Don Kayhoe was appointed as his successor. Ably assisted by John Ray and Don Hare, Kayhoe ran a program which was constantly expanding and improving for many years. So successful was our first year of operation that Congress rewarded us by swamping us with money, increasing the allocation ten-fold for the next year. Ceppellini, Dausset, and van Rood were among the first contractors, the inventory of the Serum Bank eventually filled about 10 large freezers and the collaborative program was able to contribute to the costs of running the Workshops for many years. As an offshoot it also ran a smaller serum bank for mouse reagents and held a succession of methodological and training workshops that were of inestimable value to, mainly, U.S. laboratories.

The Bank produced a series of comprehensive catalogs giving details of the method of immunization and of the specificity as stated both by the donor laboratory and by the investigators who used it. Priority for withdrawals was given to laboratories that donated to the bank or who supplied information about serum received from the bank. Start up serum kits were also provided. These covered all the common specificities and were invaluable to new laboratories.

As the program grew in size it grew in complexity. In order to monitor the program and to facilitate communication between contracting laboratories, a series of contractors meetings was held. One of the most eventful of these was held in Williamsburg, Virginia, in 1968.

Although this is getting ahead of the story, it is appropriate to mention how HLA was named since this took place at the Williamsburg meeting. HU-1 was the name proposed by the Ivanyis and Dausset at the 1967 Workshop for the system we now call HLA (23). Ceppellini forcefully objected to this term on the grounds that the symbol Hu had already been used for the blood group antigen Hunter. Most of us thought this was a frivolous objection as Hu was a rather ill-defined member of the MNSS system that had been reported as occurring in Negroes but only rarely in Europeans. Ceppellini, however, was strenuous in his opposition to calling the system Hu-1 so it became expedient to agree upon an alternative, for the spirit of unity. The name of the system was therefore debated at Williamsburg. Several alternatives were proposed, most were eliminated. HLA as proposed by Terasaki was the choice of the majority but there was no unanimity. As Chairman of the Nomenclature Committee, agreement came in a telephone call to me from Felix Rapaport at 12:30 a.m. after he had had several further hours of discussion with Jean Dausset. From our compromise agreement, the letters HLA were to have no meaning. They were not supposed to stem from the inclusion of LA, previously used by Payne and Bodmer for the LA series of antigens. By popular usage, HLA is now said to stand for Human Leukocyte Antigen but this occurred after the fact. The Williamsburg meeting also agreed on designations for the then accepted antigens, HLA 1, 2, 3, 5, 7, 8, and 9, the existence of two loci HLA-A and HLA-B was not then recognized or the terminology would have been different. Dausset would have preferred to have the antigen Mac awarded pride of place by being called HLA-1 but most participants preferred that LA-1 become HLA-1. LA-1, LA-2 and LA-3 thus became HLA 1, 2 and 3. Mac was given the distinction of having two "official" designations, HLA- 2 or HLA-Mac, but as Dausset correctly forecast, "HLA-Mac will never be used."

As mentioned earlier, one of the most urgent problems faced after the First Workshop was the correlation of antigenic differences shown by the in vitro procedures with graft rejection. Since grafting procedures are invasive, we wanted to leave either skin or kidney grafting as a final reference point and to establish an intermediate test or secondary standard. Three possibilities were examined in my laboratory, Rebuck windows in which antigen is applied to a glass cover slip which is then placed on an abraded area of skin, the mixed lymphocyte reaction, MLR and the normal lymphocyte transfer test, NLT.

For the Rebuck window, we proposed to call on Bill Boyle for soluble antigen, but first we would try various surrogate substances, including microbial antigens. According to published results mononuclear cells from an immune subject would migrate to the cover slip that had been coated with specific antigen. First, it was necessary to establish the procedure. We were able to locate an old dental drill and adapt it to take a wire brush and with this we produced the abrasions. Alternatively, we scraped away superficial layers of epidermis with pumice or with the edge of a microscope slide. Our technicians and students were willing to act as experimental subjects. We scraped or brushed areas of their arms to redness and then taped on the cover slips. The site was examined at intervals after 48 hours. In our hands this test proved to be a miserable failure. It required extraordinary cooperation from the subject. Producing the abrasion was usually quite painful and the tight bandage necessary was unlikely to be accepted by everyone. Then too, the published details didn't mention coverslip breakage or problems caused by drying out following the lifting of the edges of the coverslip. Plastic coverslips were unsatisfactory, so we put the drill in an anteroom and turned to the MLR. The problem with the MLR was not that it didn't work, but that it worked too well. Every combination we tested except HLA identical sibs gave strong reactions. An intellectual problem, first tackled by Darcy Wilson, was that several percent or more of the responding population in an MLR was stimulated whereas the proportion responding to nominal antigen should have been far less than 1%. We did use the test but never very enthusiastically and mainly to confirm genotyping by serology. The best results came in experiments in which we collaborated with Fritz Bach. Fritz was superb at performing the MLR but an indifferent serologist, and we were the opposite, so the collaboration was both pleasurable and profitable. Although the MLR is a most useful genetic tool, we found it to be a poor predictor of histocompatibility especially when applied to unrelated pairs. This was published in J. Exp. Med. rather to the chagrin of some of our friends, one of whom said, "But Bernard, before you can reach that conclusion you have to be able to do the test properly." I'll rest my case by asking how often the MLR is predictive of graft rejection. Anyway, we believed the MLR to be unreliable. This left the NLT, a most interesting procedure. The NLT was first reported by Brent and Medawar in 1963 as a genetic test. Parental guinea pig cells injected into F_1 hybrids gave strong cutaneous reactions while F_1 did not react against the parent; we tried to apply the NLT directly to human volunteers. To minimize the risk of spreading infectious diseases, we performed checkerboard type experiments in which each subject was a donor and then a recipient, injecting various numbers of lymphocytes intracutaneously into each of 2 sites on the forearm (18). The test was easy to perform, reproducibility between replicate sites was excellent, and there was a good dose response curve. Reactions appeared within 24 hours and usually peaked at 48 hours, histologically the reaction sites were heavily infiltrated with mononuclear cells. The area of induration was outlined with a ball-point pen, and a permanent record could be made by pressing Scotch tape against the skin and then transferring the strip to the patient's notes. Superficially these appeared to be delayed-type responses. However, delayed responses should not have occurred in unsensitized subjects, and the reactions should get stronger after immunization. To our surprise, they got less. The initial reactions did not disappear after 72 hours but often persisted for 14 days, most were visible and often palpable as erythematous patches for up to a month. Upon repeating the injection from cells from the same donor, the reactions did not appear any quicker, the lesions were usually smaller, and often disappeared within a few days leaving no trace. The difference was not because the cells injected were from an immunized donor because we included a virgin subject with each test. Some of the paradox was explained when we discovered that our responders were producing cytotoxic antibodies; the correlation between rapid disappearance of the reaction and the presence of antibody was excellent. Although we soon stopped using the NLT as an assay, we used intradermal injection as a means of inducing antileukocyte antibodies for several years after, producing many unusual and often unique antibodies (19). The antiserum MAX was one of these, produced in our biomathematician colleague against his son.

Frustrated in our efforts to get a simple functional test we fell back on skin grafting and used our panel of sera from multiparous women and lymphocyte injected volunteers in an attempt to predict histocompatibility. We recruited a panel of some 30 male volunteers, typed them with available sera, and grouped them according to ABO red cell groups. Within the A and O blood group panels, we arranged subsets of 4, designated A, B, C, and D. A and B, C and D were selected to be as compatible as possible, A and C, B and D were incompatible. Skin grafts were exchanged and we waited for the results preparatory to buying our tickets to Sweden. I don't think I've ever been quite so dismayed as when we tabulated the results. A did not reject C more rapidly than B nor did D reject B rapidly; indeed there was almost the reverse; there was a slight suggestion that the "compatible" grafts were rejected more rapidly than the "incompatible" ones (20). When we had recovered from the disappointment, we reasoned that our serum panel did not include the most important antibodies, but since we now had an entirely new set of antibodies made by injecting subject A with lymphocytes from C and D with lymphocytes from B and so on, we should try again. After the third attempt we had run out of volunteers. We had also lost most of our enthusiasm for this approach, so for various reasons, Chester and I then decided to try a new approach.

Unselected pairs of ABH and Rh compatible subjects were given reciprocal blood transfusions and were then reciprocally skin grafted. One set received fresh blood, the second, blood stored for 3 weeks, the third received buffy coat depleted fresh blood, and the fourth, buffy coat poor stored blood. This coincided with our hopes for kidney transplantation since we had opted to use frozen blood for our recipients. As expected, buffy coat rich blood did sensitize, more excitingly, the rejection times for stored depleted blood were almost significantly prolonged suggesting a state of unresponsiveness. Felix Rapaport had some concordant data so we pooled resources. To achieve significance we could recruit more subjects or perhaps we could augment the tolerance effect by giving repeat transfusions. We consulted the subjects, they all agreed to go through a second round and we collected donor blood and began the second stage. Unfortunately, one of the first subjects given a second transfusion presented himself in the emergency room on the day after transfusion complaining of hematuria. Also, unfortunately, the physician who saw him panicked and diagnosed an acute transfusion reaction. We hastily repeated the cross-match and when it again proved negative went over to the emergency room to consult with the physician. To our disgust the urine sample was bright red; the subject had acute hemorrhagic cystitis and not a transfusion reaction, but we were so upset that we discontinued all further experiments.

Chester, Ed Golub and I also devised another test, this time one for the diagnosis of rejection (21). Since the kidney undergoing rejection had a massive accumulation of lymphocytes, we reasoned that the infiltrates could include cells making natural antibodies and we could detect these in the urine. Our cold room filled with urines for testing for isoagglution levels anti-HLA antibodies. We reported on one patient whose urinary excretion of anti-B rose with threatened rejection and fell after steroid therapy but this approach failed to win support and we dropped it. Instead, we concentrated on trying to understand the antigen systems which were becoming more and more intriguing.

By the time of the Leiden Workshop, most laboratories could define 4a, 4b and MAC very well. At the time progress seemed painfully slow but in retrospect we accomplished an astonishing amount in a short time. Cross-reactivity was one of the greatest hurdles we faced, handicapping the definition of specificities and remaining so for a number of years. We tackled cross reactivity in two ways, by absorption and by recruiting Max Woodbury who loved building algorithms and solving mathematical conundrums as a collaborator. One of Max's patterns of cross reacting and shared specificity is shown in Figure 1. Frequently Donna Kostyu, Fran Ward, or Chinky Johnston or Edmond Yunis and I would work out serological patterns and test them by absorption or other procedures in the laboratory while Max would sit up all night either at the computer or building complex patterns on paper. In this way many new HLA antigens were identified.

In retrospect it is obvious that HLA is a system of closely linked loci. At the time of the Second Workshop when we had only serology as a test system and the sera were all highly cross- reactive or truly multispecific in that they contained more than one antibody, this was not apparent. No two sera were identical but any number of them gave partially overlapping results. We could often assign serum cell reactions to the categories strong (class I), medium (class II), or weak (class III). Through absorption we could show that our sera produced by immunizing male volunteers could be completely absorbed by cells from any of the 3 classes but it would take 4-16 times as many class II as class I cells to absorb out the antibody and 4-16 times as many class III as class II for complete absorption. (Yunis and I reported on this in detail for the serum BC, an anti-A3 serum (22). Evidently these sera were not mixtures although they often behaved as if they were. To try to solve the riddle of the overlapping specificities, Chester and I went to the Wrightsville Beach Biomedical Marine Laboratory near Wilmington, North Carolina, where we could be uninterupted. The Director, Ralph Braur, gave us unrestricted use of a large conference room and we pinned our data all around the room. Although we made progress, we did not come up with a simple solution and it fell to Ivanyi and Dausset to propose a single system, Hu-1 (23). van Rood had presumably known this but never said so in a simple manner, instead he reported crossing over in a family without establishing the basic segregation patterns of the system (24). Pavol and Dagmar Ivanyi and Jean Dausset did not have any family data but jumped to the right conclusion. As soon as they made the assertion, it was obvious that they were right.

In 1966, Fitz Bach and I, having studied the inheritance of leukocyte antigens independently and by different procedures, decided to pool our forces and test for segregation of the antigens in families. Karen Sullivan and Sharon Lancaster, from

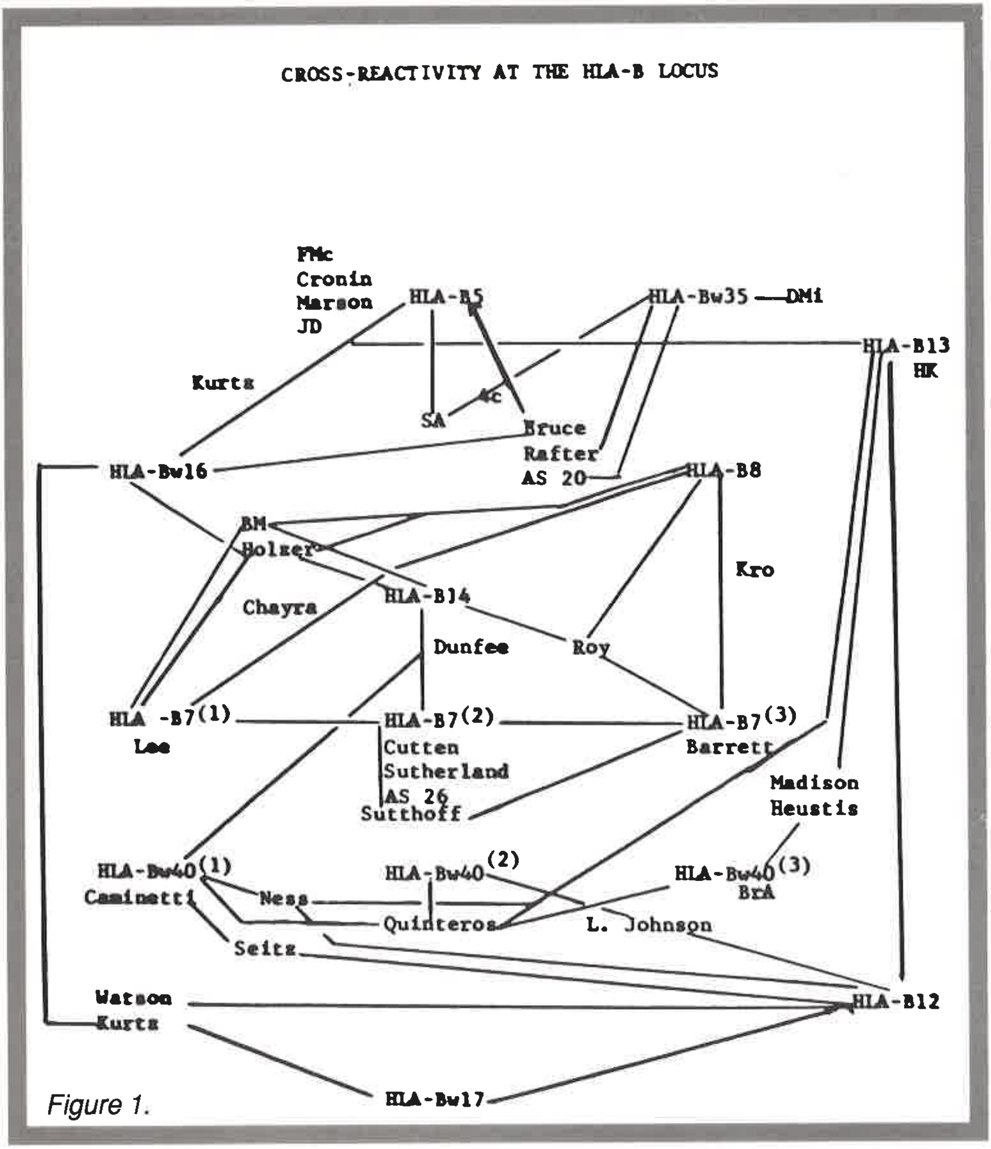


Figure 1.

my laboratory, typed several large families that had also been analysed by Fritz. One family, SA, was an analyst's dream. With our then available sera the eight sibs fell into 2 groups of 4 children (25). Six of our sera reacted 4 of the sibs and with the father, seven of our sera reacted with the father and with the other 4 sibs. There were no confounding patterns from the mother and it was obvious that we were seeing the two chromosomal inheritance patterns—(haplotypes) of the father. This was the first clear-cut segregation analysis performed, once it was done we could start to solve more complex families. The first of many of these was the Mu family. This analysis took 3 hours sitting at the table after dinner with Chuck and Janet Plate.

The IRS should support these initiatives. Scientists sometimes work most effectively in the comfort and privacy of a home and without the distractions of telephones or anxious colleagues. Fritz Bach and I first discussed collaboration in a suite at the Luxurious Biltmore Hotel in Santa Barbara during a meeting organized by Bill Longmire. Having established a sybaritic relationship in California, we later met at Fritz's house near Madison. Fritz has given his recollection of this incident.

Fritz and I published our two major papers showing the MLC reading segregated with HLA (26) (27). Once we could genotype families we could examine anomalous reactions that sometimes cropped up, for example, sera Cou and Hib would appear identical when tested on a large family except that one child failed to react with Cou. We suspected crossing over but could not prove it, but once we could genotype, it became easier to spot the recombinants. Fran Ward, Joanne Southworth and I published 6 recombinant families, unfortunately we reported these recombinants in terms of sera and not as specificities as we were not really certain of the specificities of some of our sera and the report never received the notice it deserved (28). Although our procedures were now very good and gave high reproducibility from day to day, we were bothered by the consistent observation that no two individuals in a population gave identical results. This did not easily accord with what we knew of H-2 or of any of the red cell groups. This could be due to the complexity of the systems and the outbred nature of human populations or to exogenous factors (we called them the spinach antigens meaning that dietary idiosyncrasies might affect the serum reactions) so we turned to the most accessible consanguineous population, the Old Order Amish. Victor McKensick was interested in our proposal and suggested that two of his associates, Wilma Bias and Omar Yoder might be willing to help us. Omar was an experienced field worker and Wilma, a blood group serologist and geneticist was interested in the experiments and also wanted to learn HLA typing procedures so together we worked on families in Lancaster County, Pennsylvania. For the first time we got results that could be simply explained by simple segregation.

The part played by "minor" histocompatibility antigens in human bone marrow, platelet and tissue rejection is not properly known. Severe graft versus host reactions between HLA identical siblings are common and up to 3% of HLA identical sibling kidneys are rejected, arguing for the segregation of powerful unknown antigens separate from HLA. Platelet antigens, described by Shulman and investigated by other investigators, especially by the Dutch, are not tested for by most serologists. Macrophage and endothelial antigens have been reported, but their study has been sporadic with few if any family studies contributing genetic information. In the mouse and in the rat, "pure" MHC differences between donor and recipient often give surprisingly weak reactions. Differences at single minor loci also often give weak reactions and not infrequently some of the recipients appear to become tolerized. The most severe reactions occur with a combination of minor and major differences.

We struggled for several years with the concepts of antigenic strength and also with the detection of non-HLA antigens. Antigenic strength was tested for in a transplant simulation model. Not infrequently a patient with end stage renal disease would have several potential donors, typically a mother and several siblings. An HLA identical sibling might be available for studies but not for transplantation. We then used these HLA identical sibs as surrogates to test for the immunogenicity of different HLA haplotypes and to obtain an indication of the antigenic strength of non-HLA antigens (29). We also established a mouse model from a cross of 4 different strains of mice to generate 4 genotypes in the F2 (30). From the range of rejection times between HLA identical sibs, we learned a.) that HLA and other histocompatibility antigens segregate independently in all families, and b.) that there is a wide range in rejection times due to minor H antigens; the shortest rejection was 16 days and the longest was probably 44 days. ("Probably" because this was observed in a family of 3 brothers with no parents, all 3 were phenotypically identical but could not be genotyped.) We carried out a repeat graft in this family and were surprised to find that it was rejected in a first-set manner. The interesting topic of minor H antigens is still unexplored. We did find presumptive evidence for a Y linked antigen. Grafts from male to female HLA identical pairs were more rapidly rejected than all other combinations, but the effect was weak. The Y effect was only observed when there was no HLA difference. From these family studies we also showed that some subjects reject all skin grafts more rapidly than do others (31). This is another topic awaiting elucidation, namely differences in the innate reactivity of individuals. Similar effects are seen in MLR and in cytotoxicity assays. Of theoretical interest, this unresolved problem may be of practical importance in explaining why some individuals succumb to infection while others do not. This problem may eventually be investigated in connection with AIDS; why do some subjects infected with HIV rapidly develop disease while others remain disease free although seropositive, for years.

Janet Plate, then a graduate student, made a welcome and unexpected contribution to our understanding of the HLA system. Her thesis was on lymphocyte stimulation by streptococcal antigens. Her project started as a comparison of streptolysis O and S. It soon became apparent that the strongest stimulation came from a glycoprotein we called Lymphocyte Transforming Factor (LTF). LTF was an incredibly active substance. Janet found it difficult to decide if LTF was mitogen or antigen. To gain more information we used several approaches, one was through collaboration with Yoon Berm Kim. Colostrum deprived cesarean derived piglets designated Po by Yoon and raised in isolation remain immunologic virgins, so we exposed them to LTF. The Po responded, which suggested "mitogen"—but their responses were feeble compared to the mother's and this observation suggested antigen (32) (33). We also injected LTF into our own skins. Janet, who had had a laboratory infection with the streptococcus, had a fulminating arthus reaction that was truly frightening while I, who had a long history of repeated streptococcal infections had no reaction. These observations also suggested "antigen." She observed marked differences in *in vitro* responsiveness between individuals. Janet tested family members to obtain genetic information about responsiveness. During these experiments to confirm genetic identity she set up MLR and to our surprise, two of the children who were clearly HLA identical were mutually strongly stimulatory. Janet's conclusion—which was the correct one, was that another locus, closely linked to HLA but separable by recombination was responsible for MLR (34). This was also the conclusion of Bach et al. (35) and Yunis et al. (36) in other experiments but it was some time before an MLR-locus, which we called MLR-S (now HLA-D) was accepted. Yunis and I also postulated an MLR-R responder locus and separate loci called HDR-S and HDR-R for stimulation and response locus for delayed hypersensitivity (37). Although the stimulator loci are accepted, the responder loci have received much less attention.

The 1967 Workshop held in Torino was in many ways the most remarkable. By that time Fritz Bach and I had clearly established the segregation of functional activity together with the segregation of serologically identifiable haplotypes and my laboratory had successfully genotyped many of our transplant families and were using the information clinically to optimize kidney graft survival for sibling grafts.

The Torino Workshop was designed by Ceppellini to test our ability to genotype a number of families. His assistants went out into the villages around the city to bleed some of the larger families. The samples were coded and a dozen or so blood samples were given out each morning. By afternoon we had typed the cells and handed in the reactions. The next day we were given the family relationships and were required to define the haplotypes. Several new laboratories participated. The bench work in unfamiliar surroundings was exacting and as an additional handicap there were endless banquets at night, each with an address by a local dignitary. Although arduous it was enjoyable and the team leaders were able to make rapid progress with the final analysis in the beautiful surroundings at Aosta.

This marked the end of the first phase of HLA which began in Paris with the description of Mac, switched to Holland with the discovery of 4a and 4b and ended in Torino with the acknowledgement of the universality of the inheritance of the haplotypes. Tissue typing was no longer an art but had become part of the science of genetics. In its third phase, HLA was to become the center of the universe for immunological reactivity as had been dimly foreshadowed by the functional studies already alluded to.

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Unofficial Biography

Dennis Bernard Amos – known as Bernard

PERSONAL

Born: April 16, 1923, Bromley, Kent, England, now part of Southeast London

HLA haplotypes:

a = A1, Cwx, B7, Bw6, DR2, Dw2, DQw1, Dpw4

b = A2, Cwx, B51, Bw4, DR2, Dwx, DQw1, DPw2

Marital Status:

Married 1949 - Solange Labesse

HLA haplotypes:

c = A30, Cwx, B44, Bw4, DR7, Dw7, DQw2, DPw1

d = A26, Cwx, B38, Bw4, DR9, Dwx, DQw3, Dpw4

Children: Susan (bc), Martin (bc/d), Christopher (bd), Nigel (ad), Irene (bd)

Married 1984 - Kay Packer

EDUCATION

Raglan Road Elementary and Bromley County Grammar School, Sir John Cass Technical Institute, Chelsea Polytechnic, Guys Hospital Medical School, London

Qualifications:

A.I.M.L.T., M.R.C.S., L.R.C.P., M.B., B.S., M.D. (Pathology)

TECHNICAL AND PROFESSIONAL EXPERIENCE

1939-40	Laboratory Assistant, Welcome Physiologic Research Laboratories, Beckenham, Kent
1940-45	Research Assistant, Radcliffe Infirmary, Oxford
1945-46	Laboratory Technician, Microbiological Laboratory, Harly Street
1951-52	House Officer, Guys Hospital
1952-55	Fellow, Department of Pathology, Guys Hospital
1955-56	Visiting Fellow, Roswell Park Memorial Institute, Buffalo, NY
1956-62	Associate and Principal Cancer Research Scientist
1960-62	Associate Research Professor, University of Buffalo
1962-90	Experimental Surgery, Duke Medical School

HONORS

Golding Bird Prize Bacteriology

Leonard Lubbock Gold Medal and Prize

Beaney Prize in Pathology (Special Award)

Research Career Award (National Institutes of Health)

Albion A. Bernstein Award

Netherlands, Red Cross (Presentation)

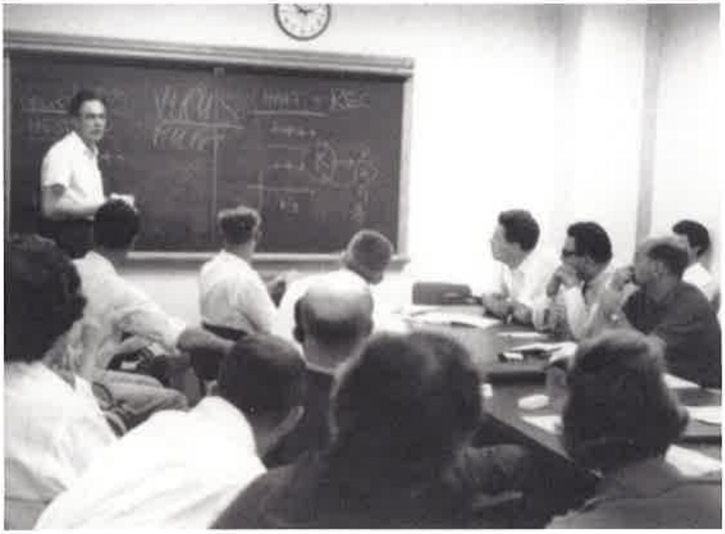
Australian Red Cross (Presentation)

	Chiba University, Japan (Presentation)
	3M Life Sciences Award
	National Academy of Sciences, Member
	Institute of Medicine, Member
	Professor Honoris Causa, Universidad Federal Do Parana
	Rose Payne Distinguished Scientist Award
1963-67	SEOPF/SANDOZ Award given annually in name of D. Bernard Amos Chairman, Collaborative Program on Tissue Transplantation and Immunity (NIAID)
	Chairman, Nomenclature Committee on Leukocyte Antigens (WHO, IUIS)
1968	Elected Past President, Transplantation Society
1965	President Second International Tissue Typing Workshop, Leiden
1972, 1977	Chairman, 1st and 2nd U.S.-Japan Symposia on HLA
	Contributor, "Ten Lives", P.I. Terasaki (Ed.)

HOBBIES

Oenology, Ornithology, Photography, Gastronomy

First Workshop Durham, 1964



Bernard Amos making sure we understand him

Nancille Peacock Jon Van Rood Bernard Amos

with concentration the test will work

First Workshop Durham, 1964

Jon van Rood

Ruggero Ceppellini

Rose Payne

Parviz Lalezari

Ruggero Ceppellini

Paul Terasaki

Leslie Brent *Kurt Hirschorn* *Paul Russell*

Parviz Lalezari, Felix Milgrom

First Workshop Durham 1964

Paul Terasaki and microtest

Parviz Lalezari

Leslie Brent

Jean Dausset

Henry Wilson

Jacque Colombani

Mme Colombani

Parviz Lalezari

Ruggero Ceppellini

Paul Terasaki

First Workshop Durham 1964

Felix Rapaport

I can do this with one eye.

Ray Mark (Crew Cut)
Felix Milgrom
Ruggero Ceppellini
Chester Zmijewski
Doyle Graham
Leslie Brent (seated)

Center: Jean Dausset, Ray Mark Doyle Graham (now Dean of Duke Medical School) far right

Brack Hattler Boyle Richard Glassock Ruggero Ceppellini

Bus to somewhere

Ruggero Ceppellini *Jean Dausset*

Paul Terasaki

Rose Payne *Ruggero Ceppellini*

Jon van Rood

History of HLA: Recollections of A Golden Age

Walter and Julia Bodmer

Address: Imperial Cancer Research Fund Laboratory
Lincoln's Inn Fields
P.O. Box No 123
London WC2A 3PX
England

Walter Bodmer
1966

Julia Bodmer
1965

1990

1990

HISTORY OF HLA : RECOLLECTIONS OF A GOLDEN AGE

WALTER AND JULIA BODMER

INTRODUCTION

The Beginning

We entered the world which was to become HLA when we met Rose Payne a few months after our arrival in Stanford in the summer of 1961. Walter, having studied mathematics as an undergraduate, had done his Ph.D. in Cambridge with R.A. Fisher, the great statistician and geneticist. There he had learned about statistics, 2 x 2 tables and χ^2 and was enormously impressed by Fisher's description of his elegant interpretation of the Rhesus blood groups. After completing his Ph.D. and just before going to Stanford, he carried out one of the first comprehensive analyses of the interaction between linkage and selection as applied to models of two closely linked loci. The theory of this interaction underlies the interpretation of linkage disequilibrium, later called gametic association, which provides the interpretation for population associations between phenotypes determined by alleles at closely linked loci.

Julia had studied Politics, Philosophy and Economics in Oxford, with an emphasis on statistics and had worked as a statistical research assistant for a distinguished economist in Cambridge. Our third child was born just a few weeks before Julia went to Stanford in August of 1961, Walter having left the family a few weeks earlier to precede them to Stanford, taking in the Cold Spring Harbor phage course on the way. She was at first abandoned at home with three small children without any obvious possibility of a job or interest outside the home. However, soon a solution was in sight!

On February 15, 1962 Walter heard Rose Payne talk. about her new white cell blood groups and he sought her help in giving his genetics course to the medical students in the Spring of 1962. Rose quickly recognised the value of having a tame statistician and geneticist to analyse her mass of somewhat incomprehensible serological data and thrust into Walter's willing hands van Rood's recently received thesis in which he had defined the first two allele system, 4a 4b, using the red cell blood group approach of 2 x 2 analysis.

Learning molecular biology left no time spare for Walter to carry out the hard work involved in the sorting and analysis of Rose Payne's intriguing and challenging body of data. Thus, Julia, driven to part-time work by the frustration of being enclosed at home in a breezeblock hut finally undertook to carry out some of the required analysis starting in February of 1963, initially even without salary. That, however, was soon remedied by a joint grant which Walter held with Sam Karlin for population genetic analysis which supported Julia effectively for the rest of her time in Stanford.

The First Groups

Walter had first learnt computing in a vacation job in the summer of 1956 with an aircraft manufacturer and then used a second generation computer, EDSACII, in Cambridge during his Ph.D. to analyse complex population genetic models. This experience made it easy to suggest how to systematise the analysis of large numbers of 2 x 2 associations for the clustering of serum reactions using the excellent computing facilities at Stanford University. Julia Bodmer, new to computing, took a course in BALGOL and then wrote her first computer programme to analyse Rose's serological data. Out popped three clusters, one of which we identified as van Rood's 4a and the other two of which we named LA1 and LA2. LA2 and LA1 were always referred to by Rose as Lukes and Gillespie because of their complementary patterns of reaction and Mrs. Gillespie continued to produce the best LA1 antiserum for many years and for eight International Histocompatibility Workshops!

These were exciting times when simple analysis of data, obtained using 'drop' serology with what Amos called 'pre-civil war' pipettes could be seen to be leading to the definition of a whole new blood group system on the white cells. Some simple theory sorted out the difference between positive and negative associations due to shared antibodies in sera and to antibodies against allelic products. This, coupled with some simple analysis of family data and a few absorption studies, laid the basis for our first paper with Rose Payne in the Cold Spring Harbor Symposium of 1964 (Payne et al 1964) and the definition of what we then called the LA locus, which later became HLA-A. While Walter was delivering his paper at the Cold Spring Harbor Symposium, (the first on human genetics), Rose was attending the first of the HLA Workshops organised by Bernard Amos in Duke. And that's why that first workshop is the only one which Walter did not attend.

The Birth of HL-A

LA2 (later HLA-A2) turned out to be similar to the first antigen "MAC" that Jean Dausset had described in 1958 (Dausset 1958). Already then in 1964 we were concerned about the nomenclature and in an appendix, laid the basis for much of the nomenclature that was subsequently used for the HLA system. L stood for leukocyte, and A for the first locus, not for antigen as is so often said. The following year Dausset and his colleagues called their set of antigens Hu1 for the first human system. The combined HL-A was decided by the first Nomenclature Committee voted into office after the third Workshop held in Torino in 1967 which met, as we recall, later that year. Our recollection is of a meeting at the New York Blood Center at which van Loghem had suggested the combination HL-A. To keep the peace and separateness of the two nomenclatures, a hyphen was introduced between L and A. This was only dropped in 1975 when it was realised that HLA would have to stand for the whole genetic region within which the letters A, B, C and D were used to describe the various loci coding for the corresponding sets of antigens.

NOMENCLATURE AND WORKSHOPS

As everyone in the field of course knows, the International Histocompatibility Testing Workshops and their associated Nomenclature Committees have played a fundamental role in the development of the system. They have kept together in a most extraordinary way an initially forceful and idiosyncratic group of scientists who have over the years become close friends.

The common nomenclature has commanded worldwide respect and has become a model for many aspects of human genetic nomenclature, which is now so important with the explosion of knowledge of human genes and of the human gene map. The reports of the Nomenclature Committee over the years are a good summary of the history and development of HLA from the first report in 1967 (Bodmer W. et al, 1967) (see note at end), which did little but decide on the name HL-A ,to the ninth report published in 1990 (Bodmer J. et al, 1990). By 1990, twenty-two HLA Class I and Class II genes had been identified and named, as had almost 150 serologically and cellularly defined allelic variants of these genes and over 150 variants identified by amino acid sequence. And more sequences are coming in every day! Nomenclature meetings, coming after several days of hard Workshop discussions, were no picnic. Discussion and scribbling on the blackboard all day and at night Rose and Walter, the most resilient members of the group, thrashing out the report to be presented to the committee next day while Julia sat half asleep in the corner. She learned her lesson there well though, when it came her time to be rapporteur (Bodmer J. et al, 1990). Small wonder that once Rose sprained her ankle falling, she claimed, off Walter's bed.

The Workshops have also proved to be a model for other collaborations in the biomedical area. They established the simple principle that when a majority of the key people in the field decide to work together, then others cannot afford not to join them. The basis for the collaboration is then to do those things which cannot be done separately by any one laboratory and to ensure that each participating laboratory gains as much from the collaboration as it gives to it.

Linked Loci

The paper that Walter wrote with Rose Payne for the Second Workshop in 1965 in Leiden (Bodmer W. and Payne 1965) set out more clearly than our first the analysis of gametic association due to close linkage, drawing the analogy with the Rhesus antigen system and applied this to the description of two new antigens 4c (later B5) and 4d (later B7) and their relationship to 4a (later BW4) and 4b (later BW6). This paper, together with our first paper on the LA system, set the scene for most future serological analysis and was further elaborated upon in a paper published in 1968 in a rather obscure place, namely the Proceedings of a Conference held in Honolulu on Computer Applications in Genetics (Bodmer W. et al, 1968). As is clear from the text, this paper was in fact written before the first Nomenclature Committee had met after the 1967 Workshop. By that time we had fully accepted the fact that the LA system and the system of antigens associated with 4a and 4b, were closely linked. The reason we had not initially suspected this was firstly because our initial data had not shown any significant association between LA1, LA2 and 4a, 4b and secondly because unfortunately the agglutination assay then used was sufficiently unreliable that several apparent recombinants in families had been found. The advantage of this separateness was that we had clearly recognised the existence of two logically separate systems (Bodmer W. et al, 1966) as systematised by Flemming Kissmeyer-Nielsen and colleagues (Flemming Kissmeyer-Nielsen et al, 1968). Our first clue that there must be close linkage between the two systems came when we saw a close association between van Rood's 7d (later B8) and our LA1. Interestingly, this association was so tight in Britain that Richard Batchelor and his colleagues in Britain originally would not accept that A1 and B8 were in fact separate antigens. Here the more mixed population of the United States of America was a distinct advantage. The contrast with the development of the H2 system is also interesting in that there the separation into D and K proved difficult because of serological cross-reaction between D and K products. Apart from the strange association between the presence of antibodies to A12 (now B12) and A9, which we first noted in the 1970 Histocompatibility Testing Workshop (Bodmer W., 1970), and which is now understood in terms of amino acid sequence similarities between Bw4, related to B12, and A9, and between A2 and B17 also related by common sequence , no cross-reactions between the antigens defined by the A and the B loci were observed.

Comparison of Assays

An important early aim of the Workshops was to compare the results obtained with different assays. Van Rood did his agglutination in the presence of EDTA while Rose Payne did not, and this difference seemed to affect the spectrum of specificities which was detected. But the real revolution in technique, of course, came with Terasaki and McClelland's development of the microcytotoxicity assay (Terasaki and McClelland, 1964). Julia, Walter and Rose therefore set up a collaboration with Paul Terasaki in which the same sera were tested by agglutination and cytotoxicity assays on a panel of 146 cells. This study, which led to Julia's first first-author paper (Bodmer J. et al, 1966) clearly established that the two assays could detect the same determinants and suggested that leaving out EDTA from the agglutination assay may give a closer correspondence with the cytotoxicity assay than was the case for van Rood's agglutination assay. By that time we had at Stanford a well-developed suite of programmes and were prepared to analyse anyone's data, especially if they gave it to us already punched in on cards. That is how we perhaps came to do the first computer analysis of Paul Terasaki's and also Bernard Amos' serological data.

Cytofluorochromasia

The microcytotoxicity assay was the obvious way to go forward and when Walter acquired new laboratories in the new Stanford Medical School building in 1966, Julia moved upstairs from Rose's laboratory and with that we started our own major laboratory programme. Our friendship with Boris Rotman had made us well aware of his elegant principle of fluorochromasia which had been applied to a somewhat cumbersome test based on embedding the white cells in agarose plates. In discussion with Ben Papermaster, who had been working on the use of fluorochromasia with Boris Rotman in Len Herzenberg's laboratory next door, it became clear that there was no reason why the principle could not be applied directly to Terasaki and McClelland's microdroplet assay system. This was one of the few times when excessive eagerness and frustration drove Walter in to the laboratory at the weekend to check it out himself and show, some time in late 1966 or early 1967, that the assay worked as described at the Torino Workshop (Bodmer W. et al, 1967). Walter carried out the assay with Millie Trip in Torino, validating it clearly in relation to the standard microcytotoxicity assay and creating amusement that an erstwhile mathematician worked at the bench and looked down a microscope. It required almost a miracle to make this participation possible, since first the sera were nearly lost when they were put in an ice box containing drinks that was taken off the flight in New York, and secondly they were stuck at the airport in Italy because of a strike which only the might of Torino's Fiat Motor Company was able to penetrate. Now, the fluorochromatic cytotoxicity assay with counter staining for dead cells, is the basis for most of the current automated cytotoxicity assay systems.

POPULATION AND FAMILY STUDIES

In the Bush

The extraordinary level of polymorphism of the HLA system, apparent even in our earliest studies published in 1964, made it an obvious candidate to use for human population genetic studies. Dausset and van Rood and their colleagues did some early population studies in 1965 and 1967, while Terasaki and his colleagues (Singal et al, 1969) did a more comprehensive study comparing Japanese and Blacks with Whites in the Los Angeles area. The difficulty with population studies was to get the white cells in a viable state from the field to the laboratory. This stimulated a search for simple media which might keep lymphocytes alive during shipment (Bodmer W. and Gerbrandt, 1968) and enabled us to do our first field study, the first HLA field study ever done, of pygmies in the Central African Republic in collaboration with Luca Cavalli-Sforza and his team early in 1968 (Bodmer

J. and Bodmer W., 1970). Oh, the fun in the bush of getting the Africans to help us by defibrinating the blood in the vacutainers by shaking them to the rhythm of the drums! Not so successful was the attempt to type the cells in the field station laboratory in the bush when the DC current to the microscope kept blowing up the fluorescent bulbs. However the transport medium we had devised for the separated cells ensured that they were viable after their long trip back from Bangui Airport in the Central African Republic to the laboratory in Stanford California. The samples were frozen and stored in liquid nitrogen and could still be re-typed nine years after they were collected. Unfortunately, a laboratory accident with a faulty liquid nitrogen tank 15 years later prevented them from being used to see whether the AIDS virus HIV could be detected at that time in the pygmy population of Central Africa. An up-date and extension of these first HLA field studies was published in 1986 (Bodmer J. et al, 1986).

In the Family

Our daughter said that once, when asked what did her parents do, she answered "They are geneticists, they breed us." Later she said that she had meant to say, had she been quite sober, that they bleed us. And so we did, in those days, paying them $3 or $5 a time, so they 'donated' willingly. Thus, we defined in the family LA-W, later to become AW31. This could be clearly defined and distinguished from AW30 because both segregated in our family since Walter is an AW31/AW30 heterozygote (Bodmer J. et al, 1970) This combination fooled Terasaki's computer in the 1980 Workshop since it only assigned AW30 to Walter and thus relegated two of our children to non-paternity status.

Crossovers

Following identification of the Four (HLA-B) and LA (HLA-A) systems as separate but closely linked, Kissmeyer-Nielsen described the first clear case of a cross-over within the system between these two loci in 1969 (Kissmeyer-Nielsen et al 1969). We defined a second case described in the 1970 Workshop (Bodmer W. et al, 1970) and speculated from these limited data on the size of the region. These estimates have remained remarkably stable over 20 years and into the era of molecular genetics, although estimates of the number of genes have changed enormously following the discovery in 1977 of introns and exons and big spaces between coding regions.

Ceppellini and Genetics

Ruggero Ceppellini was perhaps the only professionally trained geneticist, apart from Walter, amongst the early workers in the HLA field. Through this we developed a rapport and friendship over a period of nearly 25 years. His clear thinking and forceful contributions to discussions of the Nomenclature Committee helped enormously in the development of a rational nomenclature based on a proper understanding of the genetics. He had a longstanding interest in population genetics and in his critical analysis of the Gm allotypes, published in a major review written in Italian in 1967, he extended some of the formulae that Walter had developed for the analysis of two locus linked systems to the estimation of haplotype frequencies and at the same time at the 1967 Workshop in Torino he introduced the now widely used term "haplotype". The one paper we wrote together was in 1970 (Mattiuz et al, 1970) and provided a systematic analysis of the segregation of the then known determinants into two major series, and also new approaches to the analysis of segregation in families which now provide a useful basis for the extension of sib-pair analysis to test for linkage between DNA markers and a genetic susceptibility with limited penetrance. This, of course, is an important problem in the application of human genome analysis to the understanding of inherited susceptibilities to chronic diseases. Ruggero Ceppellini came to visit us in California after the Los Angeles Workshop to finish writing the paper. He had broken his arm skiing, his favourite sport, and it was fixed in a hard plaster, immobilised at an awkward angle. He was as lively as ever but nevertheless, as with so many Italians, a broken arm did seem to inhibit his speech!

HLA and Immune Response

Fritz Bach and Bernard Amos made the important observation in 1967 (Bach et al, 1967) that the mixed lymphocyte culture reaction could be explained by determinants controlled by a separate locus from HLA-A and B but one that was closely linked with a recombination fraction of perhaps 1%. Ceppellini and his group just missed making this observation but its discovery must surely have been stimulated by his discussion of genetics with Fritz Bach. A small meeting was organised in 1971 at the NIH, sponsored by the Fogarty Centre at which Ceppellini was at that time a scholar in residence. The aim of the meeting was to bring together some of the main workers in immune response in the HLA field to try and arrive at a synthesis of the current state of knowledge concerning the biological significance of the major histocompatibility antigens. Walter was assigned the task of writing on the evolutionary and genetic questions. By that time we had done our first study on the association between HLA and SLE with Hugh McDevitt, and so had been led to think about the ways in which genes in the HLA system could influence immune response, and through that be subject to selection for resistance to pathogens. This led to Walter's widely quoted review on the "Evolutionary significance of the HLA system" (Bodmer W., 1972). In this was first clearly stated the frequency dependent selection mechanism for generating HLA polymorphism, still in our view the most likely selective mechanism, as well as reasons for parallels between the HLA and immunoglobulin molecules, an idea put forward independently at the same time by Gally and Edelman. Interestingly, the notion that the histoglobulins of the HLA region and the immunoglobulins have a common origin in molecules involved in cell-cell recognition, which was implied in the 1972 paper, has been to some extent vindicated by the fact that cellular adhesion molecules such as N-CAM, I-CAM, CD2 and LFA3 and the CEA family are all members of the immunoglobulin supergene family.

Evolution

The idea that new alleles may be derived from pre-existing ones by recombination was suggested in 1966 (Bodmer W. et al, 1966) based clearly on Fisher's interpretation of the Rhesus blood group system. However, the observation that cattle alloantisera cross reacted with HLA determinants, which we first reported in 1969 (Iha et al, 1973) raised the possibility that some allelic differences may be very old. This led to the suggestion that some of the polymorphism in the HLA region may be for control of gene expression (Bodmer W., 1973), an intriguing idea that may still occasionally hold true but clearly can now be seen not to explain the age of alleles. Evolution of the region by duplication and divergence is an obvious suggestion, though at that time the suggestion that sequences may transferred between genes by some forms of unequal crossing over or non homologous gene conversion was not considered. Firm data on the age of alleles could later be obtained from amino acid and nucleotide pair sequence comparisons (Bodmer W. et al, 1986) and this idea has subsequently been developed much further by Jan Klein and Nei and others.

Immune Response and the Emergence of Class II

The discovery of immune response genes in the H2 region of the mouse and of the MLC determinants led to the search for approaches to recognising the corresponding gene products by serology. In humans, the first clue came from Ceppellini's demonstration in 1971 that HLA anti-sera could block the mixed leukocyte culture reaction and this was used as a basis for the van Rood group's (van Leeuwen et al., 1973) description of the first antisera detecting determinants on B cells which formed the initial basis for the HLA-DR serology. In the mouse, McDevitt and others described similar antisera obtained using congenic strains of mice which were identical for H2K and H2D but differed in immune response. This approach was not, however, possible in humans and the initial serology described by van Rood and his colleagues was cumbersome. In 1973, in our laboratory, we suddenly realised that the extra reactions we were seeing in cytotoxicity assays with conventional HLA antisera on B cell derived lymphoblastoid cell lines were probably against B cell determinants which corresponded to the MLC types. Similar extra reactions had been described when typing B cell derived chronic lymphocytic leukaemia cells. We had the added advantage of being able to screen sera on the Burkitt lymphoma- derived Daudi cell line which lacked surface expression of the conventional (now Class I) HLA antigens. Our first report of the serological identification of human IA-type antigens was in 1975 (Jones et al, 1975) and appeared simultaneously with similar reports by Winchester and by several others at the 1975 HLA Workshop in Aarhus. It was there that the fateful decision, for us, was made to hold the next and Seventh International Histocompatibility Testing Workshop and Conference in Oxford in 1977 with the aim of sorting out the B cell serology. Little did we realise at that time what an enormous effort was involved in such an undertaking and Walter took a sabbatical year spent mostly in Oxford specifically to help with the organisation of the Workshop and its subsequent analysis and publication.

THE SEVENTH WORKSHOP

This was the first Workshop in which the world was regionalised. We recall very clearly how quickly the Japanese region materialised following a slight hint to Paul Terasaki that it might not yet exist, and the requirement to have two regions in Italy since at that time Ceppellini and Ferrara were incompatible (G.B. Ferrara and Ruggero Ceppellini were later reconciled and G.B. provided enormous help to Ruggero in his final illness). There were more laboratories than ever to deal with and agreements had to be reached on some form of standardisation of the B cell typing. Notwithstanding that, quality controls and the exclusion of much data were needed to obtain the results that led to the definition of DRw1 to 7.

A Workshop is A Family Business

There was a time when six Bodmers were working for the Workshop, Walter and Julia, their three children and a niece. There is nothing like a family business when it comes to tight controls and hard work! Who else but a son, (Charles) would stay in a small room for many hours photocopying papers, a task that several others had abandoned after half an hour because of a blinding headache? Who but another son, (Mark) would take over the running of the University computer over a Bank Holiday weekend, churning out the analyses which his mother (and father) would later work through. And a daughter, (Helen) and niece. (Sylvia) who would sort and stuff envelopes with the results of all these efforts? The newsletters that Julia typed out on her little Adler typewriter during the run up to the

Workshop ensured, we hoped, that people slaving away over their hot benches realised that they were in good company and that results were and would continue to come out. The piles of computer output that Julia toiled through to find the magic clusters were transformed into microfiches and shipped around the world. We had had our fill of shipping paper in the 1972 and 1975 Workshops. People really never took to microfiches, floppies are better, what will be next? Obviously electronic mail. We soon learnt that camera-ready copy meant that you did all the editing and type-setting yourself and that editing also meant that you had to write many of the joint reports.

After the Storm

To recover from Workshop fatigue we took a long trip to Australia at the end of 1977. But we do believe that, after all, it was worthwhile and, as in the case of so many other of the Workshops, represented a major step forward in the definition of the system. Julia's work with Allan and Hilary Hill of the Department of Rheumatology at Stoke Mandeville Hospital led, during the Workshop, to the first confirmation of the association between rheumatoid arthritis and HLA by serology, showing a highly significant increase of the preliminarily defined antigen WIA4, which became DRW4 after the Workshop (Bodmer J. et al, 1977)

Haplotypes Across Europe

A very useful spin-off from all the effort of the Workshop organisation was the availability of a large amount of population data on many different ethnic groups. Julia took advantage of these data to analyse haplotype frequencies across Europe, realising that haplotypes, even of the two locus type, were much more sensitive measures of population differences and movements than single allele differences. The report (Bodmer J., 1980) was first given to a group of human, non HLA geneticists on an island off the coast of Finland. Not many of them perhaps understood the details but the method has proved to be very informative.

HLA and Disease

The first suggested association between HLA and disease was reported by Amiel for Hodgkins Disease in the 1967 Workshop (Amiel, 1967). He carried out this study after having spent six months in our laboratory at Stanford learning to do HLA typing at the behest of his mentor, Mathe. But nobody believed him because the data were sparse and the significance limited relative to the number of antigens tested. Only with much further testing (Morris et al, 1972), analysis of families, and more recently DP types (Bodmer J et al, 1989) has the association been substantiated and may it subsequently be elucidated.

Linkage Disequilibrium or Gametic Association

Hugh McDevitt came to Stanford in 1966 having just shown the existence of immune response genes in the H2 region of the mouse. He wanted to do a study of the association between HLA and autoimmune disease, specifically systemic lupus erythematosus (SLE) and having found that Rose Payne was too expensive, came to us. Thus started our first study on the association between HLA and disease showing a significant association between SLE and B15 as well as B8 (Grumet et al, 1971) and so our interest in the interpretation and analysis of HLA and disease associations through gametic association or linkage disequilibrium. This idea was first clearly put forward in a talk given to an international symposium on Hodgkins disease at Stanford in March 1972 (Bodmer W., 1973). There it was pointed out that if the association was not with the antigen in question but with an allele at a closely linked locus, then this must be due to gametic association and in addition it was pointed out that there could be major genetic factors in a disease that showed no familial concentration. This is because low penetrance, in the genetic sense, means that there is a small probability that two or more individuals with the same genotype in a family will all have the disease.

Review papers by McDevitt and Bodmer (McDevitt and Bodmer W., 1972; McDevitt and Bodmer, 1974) summarised the then available though limited data on HLA and disease associations and discussed possible mechanisms, in the light of data on immune response and susceptibility to viral leukaemogenesis controlled by genes in the mouse H2 region. We used measures of linkage disequilibrium or gametic association (Bodmer W. and Bodmer J., 1974) to estimate the disease gene frequency under different assumptions, an approach later extended by Walter in collaboration with Glenys Thomson.

Disease in Families and Sib-Pair Analysis

At that time, in the early 1970s, we initiated a study with Lorber and Bobrow on the families of individuals with Spina Bifida to look for an HLA association. This was the first of our HLA sib-pair studies and we believe the first to be fully reported, though it was negative (Bobrow et al, 1975), (see also Hors J. and Dausset J., 1974 for an initial early discussion of HLA and sib-pair analysis). The first positive evidence from sib pairs for HLA linkage was for juvenile onset diabetes (Cudworth A.G.M. and Woodrow J.C., 1975). Following the approach of Day and Simons (Day and Simons, 1976) to analysing multiple case families, Walter and Glenys Thomson expanded these approaches and developed the theory which has been widely used and subsequently developed by many others (Thomson and Bodmer W., 1977). This approach to linkage analysis harks back to Penrose's sib-pair method published in 1935 and is the basis for a general approach to the use of affected individuals in pedigrees for looking for linkage. This is now strongly promoted, with the extensive availability of polymorphic DNA markers, for the study of susceptibility to chronic diseases associated with significant, though substantially less than 100% penetrance genetic effects.

Our own major study on diabetes and HLA in families started at the time of the Seventh Workshop in Oxford, around 1976 and took so long to complete that it effectively became a prospective study (Winearls et al, 1984). It did suggest a clear involvement of DR1, already hinted at by our data reported at the time of the Workshop in 1977, and showed very clearly that at least the DR4 association could not just be due to DR4. This, together with the observation made by Svejgaard and Ryder in 1977 that there was a strong association with DR3/DR4 heterozygotes and the fact that DQα is the only significantly polymorphic alpha chain, led to our suggestion that the diabetes association was probably with DQ rather than DR. This is an idea that subsequently received substantial support from the work of others notably through the observation of McDevitt and others on the association with aspartic acid versus non aspartic acid at position 57 of the DQβ chain.

BACK TO ENGLAND

Somatic Cells and Biochemistry

In 1970 we had returned to England, to the Department of Genetics in Oxford University. There, shortly after arriving, we remember trying out Boyum's remarkable technique for separating lymphocytes using ficoll-triosil, the procedure he described in 1968 (Boyum, 1968). The experiment worked the first time, a truly rare event.

There we pursued the somatic cell genetics of the HLA region and β2 microglobulin and showed that the gene for β2m was on chromosome 15 and not in the HLA region (Goodfellow et al, 1975). The late Rodney Porter had introduced Walter to Michael Crumpton who at that time was in the Biochemistry Division of the National Institute for Medical Research, London. This led to an extended collaboration on the biochemical analysis of the HLA determinants and the first isolation of the complete molecule in detergent and the demonstration of its membrane association (Snary et al, 1974) as compared to the very effective water soluble papain product that had been pioneered by Nathanson and Davies.

HLA Expression

Abortive attempts to isolate HLA antigens from placenta, a readily available source of tissue, eventually made us realise that the trophoblast lacked expression of HLA-A B C antigens (Goodfellow et al, 1976), an observation made independently at about the same time by Page Faulk. This led to our suggestion that the lack of HLA class I expression on the major population of trophoblasts was the explanation for the survival of the foetus as an allograft. It also led to our interest in the role of the lack of HLA Class I expression in tumour cells as an indication of their escape from immune response (Arce-Gomez et al, 1978). Recently, Mark Smith and others in Walter's laboratory carried out a more detailed analysis including the description of allelic losses (Smith et al, 1989).

Monoclonal Antibodies

A further major development during our time in Oxford was our involvement in the characterisation and production of monoclonal antibodies to HLA determinants. This started with a collaboration with Cesar Milstein and Alan Williams and their colleagues characterising antibodies they had produced to human tonsil cell preparations. These included the now famous W6/32, which we showed, using somatic cell hybrids, was directed against a determinant common to HLA-A, B and C (Barnstable et al, 1978). Soon after this came the first description of a polymorphic monoclonal antibody, to A2 [and as shown later to AW69, the first antigen defined by a monoclonal antibody, (Parham and Bodmer, 1978)]. A wide range of monoclonal antibodies especially to the HLA-D region products, was produced by Frances Brodsky, Peter Parham and Colin Barnstable in our laboratory (Brodsky et al, 1979). These have been widely used subsequently for immunochemical analysis, including two-dimensional gel precipitations which helped define the range of protein products coded in by the HLA-D region (de Kretser et al, 1982). Another valuable application of monoclonal antibodies was to cell separation where Theonne de Kretser developed the first bead based system using monoclonal antibodies for B cell separation (de Kretser et al, 1980) . This is now the basis of the DYNAL magnetic bead separation based on Ugelstad and Vartdal's magnetic beads. This system was first used to separate tumour cells in bone marrow for autologous transplants in an ICRF Unit in London but when we suggested a collaboration to extend the method to B cell separation, they took the idea elsewhere.

Molecular Analysis of HLA

An important development that took place just as we moved from the Genetics Department in Oxford to The Imperial Cancer Research Fund in London was, of course, the application of recombinant DNA techniques to the cloning of the genes of the HLA region. Janet Lee and John Trowsdale in Walter's laboratory in London obtained the first clone for a major histocompatibility Class II product, namely DRa (Lee et al, 1982). This analysis also for the first time conclusively showed that the DRα chain mapped to chromosome 6, and so presumably in the HLA region. Soon after that followed the analysis of the alpha chain sequence showing that it was a member of the immunoglobulin supergene family and revealing its intron and exon structure and the probable domain organisation of the alpha chain (Lee et al, 1982). Next came the demonstration of DQα polymorphism, using southern blotting and restriction fragment length analysis, and its association with DR serology (Trowsdale et al, 1983) and then recognition of other alpha chains and their polymorphism (Spielman et al, 1984).

This expansion of knowledge of the HLA region at the molecular level has exploded since those early years, revealing many more genes than were at first suspected, not all of which have anything to do by any means with immune response. There is also the development of techniques for manipulating the genes to study their function and new approaches to making monoclonal antibodies (Heyes et al, 1986) in Julia's laboratory and, even more importantly, of functional analysis in transgenic mice. Now one can envisage obtaining the complete nucleotide sequence of the 3.5 to 4 million bases of the HLA region. Who would have thought that a little simple serology and statistical analysis would in a quarter of a century take us to our present extraordinary state of knowledge of the HLA region, its genetics, its products and its functions?

MODERN HISTORY AND THE FUTURE

It seems right to end the early history of HLA in the early 80's. In Oxford the modern history taught in the 50's ended in 1914, an acknowledgement of the fact that events and discoveries need time to be shaken into perspective. To hear about the exciting explosions in knowledge in the last five to ten years we will have to wait for a sequel to this volume in 25 years time.

Note: Authors are referred to in text as 'Bodmer W.' or 'Bodmer J.' to avoid confusion.

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CURRICULUM VITAE

Walter F Bodmer

PERSONAL

Born: 10 January 1936 - Frankfurt am Main, Germany
Nationality: British

EDUCATION

1956	B.A. - Cambridge University
1959	Ph.D. - Cambridge University

HONOURS & APPOINTMENTS

1972	Foreign Honorary Member, American Academy of Arts and Sciences
1974	Fellow of the Royal Society
1981	Foreign Associate, US National Academy Sciences
1981-82	Vice-President, Royal Institution
1981	Hon. Fellow, Keble College, Oxford
1984-85	President, Royal Statistical Society
1984	Fellow of Royal College of Pathologists
1985	Hon. Fellow, Royal College of Physicians
1985	Hon. Member, American Association of Immunologists
1986	Knight Batchelor
1986	Hon. Fellow, Royal College of Surgeons of England
1987-89	British Association for the Advancement of Science 1987-88 President 1989- Vice-President
1987	Laurea 'Honoris Causa' in Medicine and Surgery, University of Bologna
1988	Hon. D.Sc. University of Bath
1988	Hon. D.Sc. University of Oxford
1989-90	President, The Assn. for Science Education
1988-	For. Member, Czechoslovak Academy of Sciences
1988	Hon. Member, Alpha Omega, Alpha Society, USA
1989	Member, Academia Europaea
1989	For. Member, American Philosophical Society
1989	Ch'man of Trustees, British Museum (Natural History)
1989	Hon. Member, British Society of Gastroenterology
1989	Trustee, Greater Manchester Museum of Science & Industry
1989-	Hon. Fellow, Clare College Cambridge
1990-	President, Human Genome Organisation
1990-91	President, Br. Society for Histocompatibility and Immunogenetics

1990-	President, Organisation of European Cancer Institutes
1990-	Vice-President, Parliamentary and Scientific Committee
1990-	Chairman, Committee on the Public Understanding of Science
1990-	Hon. Vice-President, Research Defense Society
1990-	Fellow, Int'l. Institute of Biotechnology

AWARDS

1980	The William Allan Memorial Award (American Society of Human Genetics)
1982	The Conway Evans Prize (Royal College of Physicians/Royal Society)
1983	The Rabbi Shai Shacknai Memorial Prize Lectureship in Immunology and Cancer Research
1984	The John Alexander Memorial Prize and Lectureship, University of Pennsylvania Medical School
1985	The Rose Payne Distinguished Scientist Lectureship (American Society for Histocompatibility and Immunogenetics)
1987	Ellison Cliffe Lecture and medal (Roy.Soc.Med.)

RESEARCH & PROFESSIONAL EXPERIENCE

1958-60	Research Fellow, Clare College, Cambridge	
1961	Official Fellow, Clare College, Cambridge	
1960-61	Demonstrator, Department of Genetics, Cambridge University	
1961-70	Department of Genetics, Stanford University School of Medicine, Palo Alto, California	
	1961-62	Fellow, Visiting Assistant Professor
	1962-66	Assistant Professor
	1966-68	Associate Professor
	1968-70	Professor
1970-79	Professor of Genetics, University of Oxford	
1979-	Director of Research, Imperial Cancer Research Fund, London	

PUBLICATIONS

Total number: 394

CURRICULUM VITAE

Julia Gwynaeth Bodmer (nee Pilkington)

PERSONAL

Born: 31 July 1934

Nationality: British

EDUCATION

1953-56	Lady Margaret Hall, Oxford University reading Philosophy, Politics and Economics with Statistics as a special subject
1956	B.A. (II)
1956-57, 1958-59	Statistical Assistant to W.B. Reddaway, Director, Department of Applied Economics, Cambridge University
1960	M.A.

POSITIONS

1963-70	Research Assistant, Senior Research Assistant, Department of Genetics, Stanford University School of Medicine, CA 94305, USA
1970-79	Research Officer, Genetics Laboratory, Department of Biochemistry, University of Oxford
1979-	Senior Research Scientist, Imperial Cancer Research Fund, Lincoln's Inn Fields, London Head - Tissue Antigen Laboratory
1980	D.Sc. University of Oxford

PUBLICATIONS

Total number: 123

Mark, Helen , Charles
Bodmer 1965

Helen, Mark ,Charles
Bodmer 1965

Julia Bodmer separating pygmy cells to the drum rhythm

Cold Spring
Harbor, 1964

Cavalli
Sforza

Walter
Bodmer

Arno
Motolsky

Maurice
Landy

Roy
Walford

Rose
Payne

Jon
van Rood

Z.
Trnka

Seventh Workshop, Oxford, 1977

Hilliard Festenstein

Kissmeyer-Nielson

Walter Bodmer
with some tricks
up his sleeve

Julia

Frances
Ward

Sylvia
Lawler

Richard
Batchelor

Seventh Workshop Oxford, 1977

The French team
Laurent Degos
Jean Dausset
Liliana Legrand

Marc Fellous

Mariline Sasportes

Jon van Rood,
Nicole Suciu-Foca
Paul Reekers

Hilliard Festenstein
Iris Festenstein

Seventh Workshop, Oxford, 1977

Peter Klouda Sylvia Lawler Rodney Harris good old British food

A toast from the chairman

Paul Terasaki Bo Dupont

Seventh Workshop Oxford 1977

Ruggero Ceppellini

Alberto Piazza

Erna Moller with friend

Roy Walford and Friend

Dancing at Blenheim Palace

Julia with Friend

L. Cavalli Sforza   Walter Bodmer   1966

Kissmeyer   Peter Morris   1980

Daniel Cohen   1987

Julia Bodmer   Paul Terasaki
1980

Ninth Workshop
1987

Edmund Yunis Edgar Milford

John Hansen
at WHO nomenclature
meeting 1987

Rene Duquesnoy Ieke Schreuder Judy Falk Edmund Yunis Chinky Johnson

Ninth Workshop
1987

Paul Terasaki   Ekkehard Albert   Bernard Amos

Erik Thorsby   Kissmeyer-Nielson

Jean Dausset   Bo Dupont

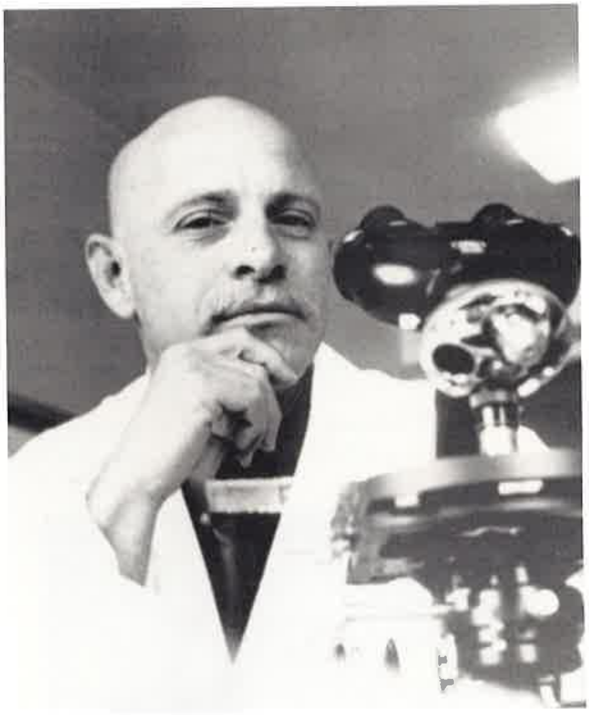
Kissmeyer Nielson   Min Sik Park

Paul Terasaki   Ekkehard Albert   Arne Svejgaard   Ieke Schreuder   Soo Yang

First Meeting WHO Leukocyte Nomenclature Committee
New York, September, 1968

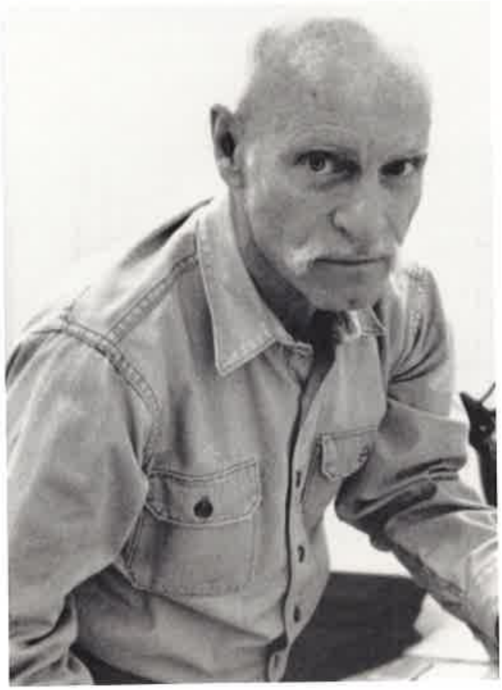
Roy L. Walford

Address: Department of Pathology
UCLA School of Medicine
10833 Le Conte Ave
Los Angeles, California 90024-1732



Roy Walford 1967

1988



FIRST MEETING WHO LEUKOCYTE NOMENCLATURE COMMITTEE NEW YORK, SEPTEMBER, 1968

ROY L. WALFORD

INTRODUCTION

It so happens that I taped a number of the meetings of the WHO Leukocyte Nomenclature Committee. What follows is a verbatim rendition of the major portion of the first meeting, which took place in New York in 1968. I have edited it, rendering out the frequent harumphs, harrahs, and interruptions, into a meaningful, and most of the time coherent script; but otherwise, it is as was said. It reflects in any case the early days of the enthusiasm and uncertainties of putting the HLA system together, and strangely enough, before any of us realized how important HLA would become for immunogenetics. Some of those present at this first meeting went on to spend the major portion of their careers with HLA – Dausset, of course, won the Nobel Prize. The careers of others went elsewhere. Nevertheless, this is how it was near the beginning. Those present at this early meeting were as follows:

Fred (Hal) Allen
D. Bernard Amos (chairman)
Richard Batchelor
Walter Bodmer
Ruggero Ceppellini
Jean Dausset
Ray Owen
Rose Payne
Jon van Rood
Ray Schulman
Paul Terasaki
Z. Trnka
Roy L. Walford

FIRST DAY

Amos: Our task is to provide an explanation of the principles on which the terminology is based, a table of the specificities, an account possibly of the immunological and genetic aspects of leukocyte and tissue antigens, and then criteria for acceptance of a new specificity, and criteria for nomenclature tied up with that. And in this respect, Trnka made the suggestion that it might be possible to set up collaborative laboratories which serve as inter-reference standards until there was an agreement on national and then international standards. The most pressing thing seems to be to decide what sort of rules we should set up for new antigens and the criteria for new antigens - how do we designate a new specificity - and which of the antigens we now recognize fit these criteria, and then go ahead and give them a designation. Is that in accordance with your thoughts, gentlemen or is there something I've left out of them? Well, to start the ball rolling let me ask our host if he will elaborate on comments he has already made.

Allen: Obviously you can define a specificity only in terms of the antiserum. And I should like to urge that the names always should apply to the antibody, not to the antigen. Everything should refer to the serum. Until one has a serum that's reasonably strong and monospecific and in large enough supply so that everybody can have some for the testing of his own panel. If all these criteria are met, then whether the thing turns out to be monospecific or just the best serum available doesn't matter so much, because you have a definite standard. And then and only then to assign an official name.

Amos: Excuse me. Shall we take that point, leaving aside for the moment the question of where it's going to be put?

Allen: Well, I think it ought to be, let's say, two liters collected over several weeks probably, and all put into the same pot so that everybody gets exactly the same material.

Walford: I agree with Hal except that there's hardly two liters of any good serum around at the present.

Allen: It isn't very hard to acquire a liter or two if you are doing plasmaphoresis.

Amos: Well, the objection will be that there are a lot of sera in reasonable quantities, say, 500 ml, that are being characterized over a period of a couple of years.

Allen: I say 2 liters; you say 1/2 a liter. Well, all right. We're still in the same ball park.

Ceppellini: I agree that the difference between the serum and an antigen should be kept quite apart. Maybe we don't know yet enough to talk about antigens. With regard to sera, microtechniques are becoming used. So I think that we could really go down on one another - I would say to 200 ml - enough to distribute to a number of recognized laboratories.

Amos: I think that it would be desirable to set the quantity very high. One might make an exception to certain very rare sera. But, if you take 200 ml - this is only enough for 10 ml for 20 laboratories.

Shulman: 200 ml is sufficient if it's very high titered.

Amos: Quite so.

Ceppellini: I am for 200.

Amos: Right, O.K. In our report we'll smooth it down to an absolute minimum of 200 ml. This is setting aside the question of what this antiserum is actually defining because we can come back to that.

Dausset: I was not clear about how to define an antigen. To define a determinant you need only one sera, if it is monospecific. I am afraid that for many of those sera, different dilutions will be monospecific. So you will have plenty too many of monospecific antisera. So, I think the best would be to make the historical step, which is to have, first of all, few sera very high coefficient of correlation. Afterward, to find a standard and to get 200 ml.

Allen: Well, I think it's fine if you can get more than one serum but I think it would be very poor to reject a good serum just because there wasn't another one like it. We would never do this in red cell grouping.

Dausset: I did not say that. I said to give an international name. You can keep your good sera, surely, and even thirty of these sera, but to give an international name!

Ceppellini: I will say we must take as a base what we know now – therefore, the historical antigens. Then make good head of the series. Also for the terminology.

Amos: Right. Now I think we're going to stick on this point. Would you reformulate this so that I can take it down?

Ceppellini: Let us give an example. From the historical point of view certainly let us take 4a which is the most complicated. 4a has an historical meaning and we know more or less what it refers to. There should be a 4, or 40, or 400 series, and that refers to a 4a serum, as presented by Dr. van Rood, or the best which is on the market at the moment. And then, some of the similar ones, let us say Torino 2 should become 42. And Dausset-3 would be 43.

Amos: But you are in agreement with Dr. Allen in saying that a particular antiserum should be given a particular name, and be quoted as either defining specificity 40 or reacting with 40. The next serum which has a high correlation with it should be given a number which will be associated with it. But it won't be given the same designation. Is there dissent to this?

Walford: I think we have to be very careful not to propagate a terminology for antigens that don't really exist. And there are some things – your letter indicated that you have some doubts about 4b – whether it's a distinct single antigen or whether it's a collection.

Dausset: But I think 4b is just as complex as 4a.

Amos: I don't think we're running into that problem.

Walford: I think we will if we name a lot of antigens and then it comes out later that there isn't really any single antigen that can be designated by the terminology, say 51. At present, in my view, we can only be sure that about five things really exist - LA1, 2 and 3, 7c, 7d and maybe a couple of others, and that's all.

Trnka: I think that at this stage in our knowledge we have to make arbitrary decisions as to what really exists, if it was several times repeated, and give also the name. Its identity is already the difference.

Rood: Well, I would like to second the motion, and come back with a misunderstood discussion very well introduced by Hal Allen, namely, that we should decide what to use as a standard. Should we, before we start, before we tackle all subjects, first get an agreement whether or not it is a good idea to have a serum as a reference? Because this is more or less a new idea.

Shulman: I think that if you're going to do it stepwise, that one suggestion was antiserum as reference, and the other was whether you need one or more, which we never arrived at. And how you determine the monospecificity of the antiserum hasn't come up yet. It's a very important point, a separate topic. Do you need more than one serum of the same specificity to call this or name it or number it? From my own experience we found that limiting the ones that we gave names to, to those having nearly identical companion antisera almost put us out of business because we found so many single specificities that in my own mind convinced are monospecific, which we haven't named at all, because we've never seen a duplicate.

Allen: Well, the comment I wanted to make is in response to Roy who says that maybe the antigen doesn't actually exist, and I think what you have to say is that the thing that really exists is the pot of serum. And you could describe its reaction. And the antigen you really don't see.

Amos: Ah, we're digressing. We're not sticking to the points. Are you sticking to the points?

Bodmer: I can't relate, on the question of monospecificity, to using a single serum. Supposing you have one serum which has an antibody, say which reacts with either/or LA1 and LA4, which I think we have for example. Then this may seem a monospecific serum by our theory of absorption, but in another population, for example, it becomes divided. So I think we have to be careful in defining new specificities on the basis of single sera, even if they are apparently monospecific. And really you can't be sure of any of this unless you know what the antigen is chemically, and if you are going to take that stand I think it's almost a semantic problem with the specificities defined from a purely logical point of view.

Amos: Well, your point is good, but it's a point for later discussion because the tone has already been set. Let's do the easy ones first – the ones where there is no question. So let's go back. Jon wants to raise a point as to whether it is correct to base our terminology on the reactions of certain sera.

Rood: I wanted to raise the point Hal Allen raised here.

Amos: Well, raise it.

Rood: Well all right. I agree with Hal and I think we can more or less decide on it here and now. We will start with a number of people who have the good serum, but we should be very clear in our minds whether or not such a serum should be defined by absorption, and also, studied in different races. I think this is an important point.

Amos: I see. I do see, but I propose to take an actual formal vote on this point of using sera to define specificities and then a second vote that a single serum shall define a certain specificity. Now we'll speak to that point.

Batchelor: Bernard, can I say one thing before you speak to it? If you have a situation envisaged by Hal Allen, then perhaps you could set a slightly different criteria for that single serum because, as Walter was pointing out, the problem of cross reactivity and so on, but in other cases you can show that the group specificities, or single specificity which it seems to be picking up, is quite different from any of the recognized ones. And therefore, under those circumstances, I think it would be fair to give it a standard number even if there is only one serum.

Ceppellini: I think it's important before taking the vote to realize what Batchelor said. We must have different criteria and I would like that you first ask us if we agree on giving some hierarchy, different importance, head of the series, how do you say?

Amos: Hierarchy!

Ceppellini: In this we simply should have a different criteria – for the head of the series. So I really would say first, if we agree on selecting a few historical complexes, and then get down to details.

Amos: All right. Jean, do you wish to speak?

Dausset: I want just to agree completely with Walter and Jon about sera. The cross-reactivity is very important. You have reactivity between antigenic halves – in fact, two identical alleles. The so-called inclusions, you know, if we can't find an explanation it will surely be a pitfall of domination. And also I'll agree with Jon's statement about populations. A serum monospecific in one population may not be monospecific in another population.

Amos: Well, as soon as it's shown not to be monospecific in another population then we're forced to take it out.

Terasaki: One other thing that hasn't been taken up yet and that is when you use any serum to define a specificity you also have to say what the method is.

Walford: I have a feeling that we're trying to be too radical in doing things. For example, if we had met six months ago or a year ago before the business about cross-reactivity was realized, as it is realized now, we could have set up a whole bunch of criteria that would have been wrong. And there are things we don't know about now undoubtedly. That's why I emphasized that we should take only about five or six specificities that are well-known, that everybody agrees on, for which there are good sera available, and name them either by the antigens or by the sera, and then stop.

Amos: I don't think there's any disagreement on that at all and the first note I've got on my paper, which I didn't read out, because I thought it was not necessary, was the danger of going too far and committing us to something which later on proved to be very unwise.

Bodmer: I'd just like to speak in open support of Roy and Ruggero. And I think that one can do this by taking the standard Ruggero says and having a hierarchical description.

Owen: At the risk of confusing things further, I think it seems to me, sitting on the outside here, that this discussion has gone from Hal's introduction where the suggestion was that what you will be designating will be antibodies, through Ceppellini's thing – where we're considering whether the determinants, not antigens – to Roy's talking about naming the antiserum. Personally, I would like to see the first part of the designation restricted to designating the reagent, recognizing that different cells get different patterns of reactions, and that there is some distance to go before you start defining the antigen.

Amos: But we are in fact taking a vote merely on the first point, and secondly, that we should use antibodies as a specificity. Could we now take a vote on that?

Walford: I would like to know of what Hal means. It isn't really very clear to me what you mean, Fred.

Allen: Well, I think what I meant, Roy, was that, take Mac, we have 100 different anti-Macs that everyone agrees to are anti-Mac, but I think the specificity that we decide on for Mac should be defined by a serum – just one serum, from one individual – or if you want a pool, a big pot of serum, more than one.

Rood: I very much agree with you on this as I said before, but what I'm thinking about is, for instance, in certain test systems you have a weak Mac reaction and a strong Mac reaction. And if we are going to use a serum as a reference we should also include in our designation a very detailed description what is a+ and what is a– reaction. And what we also should include is what constitutes a serum which reacts identically, whether this means that you have to test 100, a 1000, a 2000 samples in 1 or in 5 populations, without and with families and so on. I think all these points should all come into focus before we can take a vote, Mr. Chairman. Otherwise, you don't know what you are voting about. We can say now, all right, we take a serum as a standard reference. Right? And then we talk this through and we decide that you have to test 10,000 samples, 100 families, and absorb all your negatives – which is completely impractical and some of the people will not like it. So before we can take a vote we should redefine what we are voting about. What about it, Rose?

Payne: I agree with you, Jon, that we should define what we are talking about, for sure. But I don't think we can do all the work that is necessary to have a rigid classification and we may have to resort to something simplistic because we are not going to do 30 absortions on every antiserum.

Shulman: I think that a discussion of Mac relative to some of the other antibodies that are assumed to show the same specificity might help clarify the issue concerning what we should call an antigen. And correct me if I'm wrong at any point. Mac includes a number of different antigens in your context, Mr. Dausset, I do believe. Therefore it is not a single specificity but it is as many as 7 or 8 specificities as identified by the original antiserum.

Amos: But then, you see, we're sort of leapfrogging this in saying the original Mac is of general historical concern. And now within Mac, Jean perhaps, or Rose, may have an example of a serum which will fulfill the criteria which we will later decide. The provision of the various terminologies being suggested is largely to set aside certain blocks of numbers which would hopefully reflect this relationship.

Shulman: If that is what Hal means, that's fine, but I didn't understand him to mean that.

Amos: As far as I'm aware, you picked one of the few examples where what you say doesn't apply. As far as I'm aware, D1, Mac, and LA2, they are identical within Caucasian populations - this was shown at the last Workshop. We each used interchangeably sera that react identically and I think that's one very good case where you can define the antigen without any complications.

Dausset: But the point that I wanted to say today, we are just to choose the level of magnification that we want to give a name, because there is no end, you know, no beginning and no end. You have to choose the level of magnification to give the international name. And this is very real, I think too. And for that we need many sera. I don't think that we need only one.

Amos: Well, can we reach agreement? Should we dispose of this, that the reference will be a particular antiserum?

Ceppellini: I again don't agree. If we decide to have different levels and there is a group - because in practice, original - I don't think about Mac, which is a symbol, but also about 4a. 4a has some meaning at the moment If we decide to have a level that approximates as 4a...

Walford: I still have the feeling that if we decide to have different levels we're going to end up doing too much. If you have different levels, it's going to be very easy to do too much and we're going to be trapped.

Ceppellini: But it's just on the first level now. That's my point.

Bodmer: I think that Ruggero's point is that we should have the one level which we decide on now but decide on it in a way that later on we can have the other levels. I mean, have a system which can develop in a flexible way. And I would still say that even for Mac LA-2 I think there is more than one sera. Why not use 2 or 3 sera if you have them? The same is true for LA1, the same is true for LA2. I don't see why we should fix ourselves on one serum.

Amos: This may be true for Mac, but it may be a dangerous precedent for some of the others. If you establish a particular serum as your primary reference, you relate other things to it. And then you say, you know, this isn't defined HLA2 or whatever you're talking about.

Rood: May I come back to this question of one versus many sera? If you want to make the decision at once, you should formulate the question clearly. If you want to have reference serum which indicates something which is reasonably well established, you choose one serum. Or you go back trying to define an antigen, and in that case you must have several sera. Are we going to decide for something which can be used by all other people and recognized as something which is certainly something, like the Mac LA2, or do we want to really define an antigen. I think they are two completely different things.

Ceppellini: Not so different. First, with the serum or the group of sera you must define a reaction in a reference panel or in many reference panels and then, as a second stage, with this part of a reaction, you define other sera, and therefore an antigen.

Trnka: I think that what Allen said is extremely important. It doesn't matter what you decide, because we have flexibility in 3, 4, 5 years if we find that Mac is two different groups. So it means that we are anyhow arbitrary in all our decisions.

Amos: We are trying to get over the terrible problem everybody outside the field has of understanding us. If we can, you know, get some uniformity. Later on we'll be a little bit wiser as the antigens become known. For the moment, all you need to do is to propose a motion which will be reached inevitably.

Bodmer: I'll formulate one if you want me to. The key thing is that whatever you call it – we're putting people with respect to each name that we want to assign, into two categories, they have it or they don't. They have LA2 or Mac or whatever it is, or they don't have it. And the thing we want to define is something that more than one person can recognize and agree that it's the same entity. Whatever you call that, I call it an antigenic specificity. I don't think that implies that you know anything about it chemically or that you understand anything about the true nature of the immunological reaction. I don't think that's true of any system until you've done the chemistry of the antigen. And I think that with respect to B1 or Mac or LA2 we would agree with the classification of people into those two categories. And I would say that we could now choose at least two sera which agree identically, or maybe more sera, and use those as the basic reference sera. In other cases we may only find one.

Rood: If you have a discrepancy between these two or more sera, what are you going to do?

Bodmer: If I were asked to present a reliable level now I'd say it would be on the order of a few percent which would be tolerable at this stage of the game.

Batchelor: But the more sera you use for your criterion the greater the range allowed.

Ceppellini: It seems to me that for the sake of philosophy we are going backward. Let us take what we already know, the Torino Workshop, or if you don't think it's enough, maybe another Workshop, and let us work on data.

Shulman: I think we are all passively assuming that allelic relationships and genotypes can't really be defined very well by antisera that we have on hand in white cell series. Therefore, we can't talk in terms of real antigens and genetic relationships that are clearcut.

Bodmer: Tell me what you mean by "real antigens."

Shulman: An antigen is something that in terms of defining a locus you can give a genetic characteristic to.

Bodmer: What do you mean by a locus?

Schulman: Well, it is not always clearcut. But if you take, let's say, the co-A co-B, or the PLA1 PLA2 you get very clearcut genetic relationships within families. They happen to be biallelic. ABO is complicated but at least it's triallelic – you can do that much with it as far as defining a genetic relationship between the antigens. With the white cell antigens, you're in much more trouble. Ceppellini seems disgusted but he could perhaps comment.

Amos: No, at a later time very much so because I think we will have to go into this, but it's opening up the discussion a bit too wide at this particular time.

Terasaki: With regard to the facts of the matter, there are about 10 Macs that act almost identically. Also with regard to the point of reproducibility, we also put in this test triplicates of maybe 5 Mac sera so that we have a very good measure of how good the reproducibility is.

Ceppellini: Excuse me, but I think that again we cannot forget the work that has been done the last 10 years. What was the approach? First, to begin with some sera and define an antigen. Then to introduce the idea of the cluster and then to define a specificity inside a cluster. So we must follow this parallel line.

Amos: Yes, we are going to. I'm sure we are going to.

Dausset: I agree with you. But you must choose your populations. So you have to define the population of whites, yellows, or blacks.

Amos: Would it help to discuss instead the general proposal that's been made to set aside blocks of numbers for defining these clusters.

Ceppellini: Yes, I agree. That is my feeling.

Amos: We can fairly easily list the things that will be in agreement. The first is the B1MacLA2 antigen or antigens, in the LA2 series, and then would be LA1 LA3. We probably shouldn't discuss LA4. What do you think?

Bodmer: I think it's beginning to be sufficiently well defined.

Amos: Then, 7c? 7d? What was the other name, LA5? What did you call LA5 at the Workshop?

Ceppellini: Torino 13.

Amos: O.K. Are there any other major candidates?

Bodmer: I think you ought to also use 4a and 4b.

Amos: Well, I'm putting these over on the right hand side of the page.

Ceppellini: And my 11 is your what? Torino 11 is the antithesis of 5.

Dausset: I think we should use 4, my 4.

Bodmer: I'd like to make one comment. It's not necessary blocks of numbers – the head of the series number – that number can go on as long as you like. The first number in the number should define the series. So say 1 was reserved for MacLA2, then any subsequent antigen beginning with a 1 would be classed as belonging to that series.

Amos: The proposal that is being discussed is to say: we set aside 10 prime numbers. This in itself is not sufficiently flexible. It's going to be particularly difficult, I am sure, when we get to 4a and 4b. It's quite likely that we'll fill a fairly big box.

Ceppellini: But it may be that we are 9 Arabic numbers, and these are for the moment enough to define 9 broad categories.

Dausset: But you have also to define the size of the box.

Ceppellini: No, from 2 to infinity, from 3 to infinity.

Dausset: You want to give a name to this big box.

Bodmer: Yes, one number for anything which is related to 4a.

Ceppellini: But we have a serum which cross-reacts with 3, 4 and 5 - that's Torino 2.

Rood: Excuse me, I have to go, but I have a very general statement. About the subloci I have no opinion. I think it is rather early in the game. I personally am not biased against it or for it. I don't think I have enough data. You could use, however, as an argument to introduce subloci, that if you have two subloci you would double the number of specificities because you would have, for instance, an LA sublocus with one up to nine, and you would have a 4 sublocus with one to nine. And this would give you 18 specificities. I don't think this is very valid. I am very much concerned that we should not forget that the names you are giving to these antigens will be used by residents, surgeons, technicians, and we should try to make a nomenclature which will be as little confusing as possible.

Amos: It's fairly apparent that people really want to get some concrete examples before them. Should we not consider the specific examples, then see how the general case builds around them. If so, then we ought to discuss Mac from the historical point of view. The only reservation I've got is whether Jon should be here or not.

Ceppellini: Dausset 4, Torino 11, not 4d.

Amos: What is 4d?

Dausset: 4 is Torino 11.

Amos: Where do you put 4d.

Dausset: Put 4d? I don't know.

Bodmer: 4d is 7c. That's what we've got.

Amos: One of the parts of the report will be to make a table of equivalents.

Dausset: It is not so important to know that they are in the same series as to know that they are alleles.

Ceppellini: I don't think it's important.

Dausset: Why? Why do you disagree?

Bodmer: Because we shouldn't and can't define the antigenic system in relation to the genetic.

Ceppellini: Absolutely!

Bodmer: The devil is that when you start getting into the genetics, you don't really know what you're dealing with. You don't know whether you're dealing with one system, with a hundred systems, with one basic chemical substance modified by many enzymes controlled by a set of closely liked systems or what. I don't think at this stage one can even hope to define a system in detailed relations with genetics.

Walford: Genetics! You keep on throwing in DNA and saying that we can't do anything.

Bodmer: Classical genetics is HLA and that's about as far as we can go from what we know.

Walford: You can explain it in terms of association and ,family studies pretty well if you limit the number that you're going to work with. You cannot define it in terms of base pairs, that's true.

Dausset: I am agreed in the fundamental way that you are presenting on a genetic basis. I agree completely. The only point is for clinicians we must have a very clear nomenclature.

Amos: Well, does it help them to be told that there are subloci?

Dausset: I think that's very easy to work with.

Terasaki: To get down to specifics, what we do, suppose we call LA1LA2LA3LA4?

Bodmer: Referring to what Ruggero said, I could state now which things I think deserve to be heads of series. I think 4a and 4b do. MacLA1LA2 does. I think LA1 does and LA3 does, and possibly LA4. And I think LA4 will be more complicated than LA2. And that gives us six. I think with those six we could manage now.

Dausset: I don't understand, because , for example, A1 is in series 4b.

Bodmer: No, no, no.

Dausset: Yes.

Bodmer: LA1 has never been associated with 4b.

Terasaki: Well, I think the LA1 series is a very systematic and very practical. Right now it's LA1, LA2, LA3, and LA4 and that's very convenient. I'm only speaking, that's the way it worked. Now, if you are going to give that a number like 10, 20, and 30, you are really destroying everything.

Bodmer: I'm not sure how much significance that has.

Terasaki: You don't mind that?

Bodmer: I don't, even though I'm the initiator of it.

Walford: Bernard, could you resume the chair so that people can speak in order instead of who happens to be the loudest.

Amos: O.K. I've thought that by dropping out and letting it go, it was running more freely.

Walford: But that's why I have to shout so loud. I got all my shouting during the May days in Paris, 1968, thank you, storming the Theatre de France. We have spoken on a number of occasions about making things easy for the clinicians. If we look at what has already been made easiest, it's clear enough they're less confused by LA1, 2, 3, and 4 concept than any other. And 4a and 4b. Everything else they mix up.

Owen: Let me try something. You abrogated the decimal point a while ago because you thought it was unnecessary, but it seems to me if you put LA1 point, and then do your clusters after the point, you can deal with this nomenclature at two levels. The clinicians level may stop with 1, 2, 3, 4. You people who are dealing with factors of related reagents may use numbers beyond the decimal point. Instead of saying LA10, 20, 30, use LA1, 2, 3, 4 but put LA1 point one, LA1 point 2, LA1 point 3, to have an expandable nomenclature for differences within the cluster.

Amos: The other terminology that Ray Owen and I discussed earlier probably won't rate any favor with you, but let me drop it in. Give the specificities in order of their discovery, so that we start with 1, 2, 3, 4 and go on into a system of complete anarchy. Now this avoids the danger of people making associations which are not true associations.

Ceppellini: Excellent for research, not very useful for clinicians.

Bodmer: I think we already have a certain amount of knowledge which all of us agree on, like the allelic relationship of LA1, 2, 3, and maybe LA4, and all of us agree that there's sort of something which we call 4a and that it's sort of allelic to 4b, and that these are much associated with the others. I think that we can literally combine most of the things that have been suggested by a numbering system before the decimal point, like Ray Owen suggests, by keeping, say, LA1, 2, 3 and 4, together as the first numbers. But I don't know about LA4 and 4a. I guess that will have to be argued out. But I think that this is still keeping a lot of the structure we now regard as being flexible, and allowing developments in the future with what we already know.

Amos: That's very good. But we must wonder whether the head of series is really going to be the important specificity within that series.

Owen: I can think of many instances where within Rh, a big D woman makes anti-big D antibodies except they don't react with herself. So operationally you have a finer structure to consider.

Batchelor: In terms of kidney grafting, it may be perfectly satisfactory to get compatibility at the first level, but you might have to go down 3 decimal points to get it right to prepare for marrow transplantation. Or you may have to go only two points for liver transplantation. We could surely cover this point in the report.

Bodmer: So there we have some meaning on a quantitative level.

Batchelor: In transplantation terms you are more likely to get a more violent reaction if you stay within a single letter.

Shulman: All right.

Batchelor: I know that there will be incompatibilities.

Amos: Let's leave it and talk now in general terms about the criteria for recognition.

Batchelor: Have you accepted then the proposal of Dr. Owen?

Amos: I think this corresponds to be the general view.

Walford: Could you rephrase the proposal?

Bodmer: Can we have the proposal and have a score vote on it just to see.

Owen: Well, let me try saying what I think we've got. There should be a limited number of series recognized by the members of the series being frequently correlated. And the series distinguished from each other by lack of such correlation. And the series should be numbered with separate integers followed by a decimal point, with finer distinctions within the series then made by numbers that follow the decimal.

Dausset: I agree completely with this, but just would like to know what is the size of the series, because we can have large series extending from the point and then we can have small series right at the point of say, Mac.

Trnka: I think what you have said is very important and will be helpful for memory, because you can have a series, let us say, LA4 and 20 other positions after the decimal point. They wouldn't mean too much for transplantation, but LA2 will and everybody would know it as like a capital D of the Rh-system.

Bodmer: Well, it seems to me we can decide on the principle first, and then argue if we have the decimal point, which allows us to have more series. I don't think that the principle, which is an important and a useful one, of having this sort of a notation, should get bogged down in the decision at the present time exactly what each series should be. That's the next stage.

Dausset: But what I have proposed is to have the number point and another number for the antigenic factors and the definition would be for r to be bigger than some value.

Terasaki: You are thinking of a first number representing a sublocus?

Dausset: No, I am thinking of the locus.

Terasaki: We do know something about these antigens LA1LA2LA3LA4 and that would be lost in the mess of numbers. The same thing has happened with Rh.

Dausset: The first name that we give to the series will be the names of MacA1A2. And other names, other figures will be for subdivisions.

Ceppellini: And with 2 series, is that possible?

Bodmer: I would just arbitrarily assign a new specificity to the series with the highest r...

Ceppellini: But maybe it makes a new series?

Bodmer: I mean, 7d is an example. It has a high r with 4b because it's included within it, but it has also a high r, with LA1 – which is not a logical inclusion – it's just a high correlation. I think we'd all agree that you'd put it in the 4a series.

Ceppellini: Maybe we could have another criterion, not only a very high correlation.

Bodmer: Well, I agree that one has to be careful in using only r on a statistical basis, because I would feel that a complete inclusion, which may not give a very high r value, should satisfy the criteria. Maybe one could in fact list this as a separate criterion for putting something in such a series.

Dausset: No, I think that the one would be for Mac and the point one would be for the factor of the Mac, if it exists.

Trnka: I think that in this situation we might have several, let's say 6 or 10, collaborative laboratories who all will have the material, and the there will be agreement among these 6 laboratories as to the r-values.

Dausset: If we would now take as a suggestion that the Mac we call either LAMac or 20, I would then examine these for LA8.

Amos: At the moment I get the impression that really one of the biggest stumbling blocks is undoubtedly the question of precedence. I think your main reason for pointing to this is historical precedence. I think the reason other people are wanting other changes is because there is a pretty good sequence, you know, already established.

Dausset: But there is also my paper, you know?

Amos: Everybody has read your paper and quoted it many many times, Jean, but I also think that the introduction will make abundantly clear that Mac wasn't the specificity. There isn't any specificity called Mac.

Trnka: It's a reasonable position, and indeed let's go now further and let's leap at the proper time to think it over.

Amos: Now, assuming we are going to designate a specificity, as Hal started out today by saying, a couple of the necessary attributes of any serum that's used to define a specificity is if it's strong and present in abundant quantity. The other thing is, it should generate as little confusion as possible. I think we use the word "monospecific" with great reservation. It's only operationally monospecific. Now one of the things that came out of the Workshop is, – which sera would one consider as being suitable for definition of antigens. I was looking through here. I see there's one of Rose Payne's sera which got no positives whatsoever; so under the condition of our incubation test this one just does not presumably have time to react. On the other hand, correlation between Rose's Gillespie and Walford's Morrison is phenomenally high. It's perhaps not so high in Kissmeyer-Nielsens's serum, in comparison, but Dausset gets an r of 1. So, if you are talking of defining an antiserum that would react as an LA1, it would seem that you had two sera that would fit your probable definition of the antigen. Now that's one aspect of it. By a serum exchange like this, it seems that we could fairly quickly decide on which sera could be thoroughly relied on to give similar results under different conditions. If you didn't find that, then you would be looking probably for sera that gave a specific relationship. If you were hunting for LA1 and you had what was an acceptable LA2, then your best LA1 would be one that gave a pure allelic relationship in families. This will be the mode for new specificities that are being submitted for consideration later on.

Rood: All right, but you need more than one reference serum.

Bodmer: Well, for instance, for LA1 we type in duplicates, using our Gillespie and Roy's Morrison. And an LA1 is only defined if there is substantial consistency, which there usually is because those are two very good sera.

Terasaki: Well, let's see. You know there's about six sera that have Chi squares of 150 or 160 for LA2, and it's hard to say which one is better. It wouldn't make much difference. In the LA3 series there's Roy's Thornton and Storm; we do get a difference here. It seems to me we could create an international panel in which there's 100 people in Torino, 100 in Stanford, 100 in Paris and, as Roy did, he went there to test it but the next time around he can do it a lot easier. He can put his new sera on a plate. It would go to Dausset. He would test his same panel on the new sera, but all the old sera would be already tested so that we would know exactly what the panel consists of.

Bodmer: I think that's it. I think we should have, as Dr. Trnka suggested, a series of maybe half a dozen places where there are standard reference panels, these panels having been typed with what we consider the reference sera, and then these are the places where you want to verify new sera, the places that have to do the work to show what's not true.

Dausset: The cells of the panel must be kept in the deep freeze. If you want to compare sera 10 years later, you must have the same panels.

Bodmer: So long as you have substantial overlap with what you had before, it may not be too much of a problem.

Terasaki: So it could be suspected that we made a mistake in not finding Storm and Thornton exactly alike. But if these plates went to other people and other laboratories got exactly the same results, then I think you have to accept that. So it's a very good way in which we can compare sera, you know, even your own sera. You could compare it on Dausset's panel, on Ceppellini's panel. But I do think that the plate has the advantage in that this could be done very rapidly. It comes in one day. You could start the same day.

Bodmer: We can't use those plates. I mean, I wouldn't accept that. I would take the serum and do it my own way. I'd give you the data if you wanted it.

Amos: Ruggero just whispered in my ear that we're getting involved in technicalities which several of us are much more interested in and better able to go into than others. And this is just a sort of thing that could be decided if 2 or 3 people are writing a report.

Walford: What has come out of the present discussion is that we know pretty well that there are some well-defined factors – LA1 Mac or LA2, LA3, 7c and 7d. Whether or not we have absolutely perfect serum for those, we know that these factors exist pretty well as discrete entities. We don't know now which serum to be assigned as being the perfect serum. And so I think we have to lean a little bit in naming the specificities, with less reference to the serum, and then say these serum are among the best for detecting this specificity. But not tie up the specificity in the absolute sense with a particular serum, or even two sera.

Amos: We have to tell other people how they're going to define the specificity that we've called LA2 or whatever it is.

Trnka: Yes, and we can say that this specificity has to be defined through collaboration with such and such a laboratory.

Ceppellini: I don't know. We are talking about two different things. You are asking how can a new laboratory know if he has in his hands an anti-LA2. That's very simple. As we have taught for the last 2 or 3 years in immunogenetics, he must have a panel, must build up his panel through the sera of the serum bank.

Trnka: We have a serum bank in the NIH which sends out 5 cc samples. But I don't think that we should take this into account. But once you will nominate, let us say, 7 or 8 laboratories then the other totals would be established on the basis of the panels of these laboratories. But the panels of these individual laboratories have to be worked out through the collaboration. This is a moving process with the panels in these laboratories, and we have the confidence that these laboratories have such a reputation and experience that they can insure that the new panels will be correct. Because they will then say, "Give the approval for that."

Amos: Well, so we come back to the point that the first responsibility is the laboratory that is claiming that this is a new specificity. And then the definition of other standards can await the results of that laboratory going to a trial.

Trnka: But we will have laboratories in 7 different countries. So we can nominate these laboratories here by this committee, and that we have a new specificity I think should be agreed by all these seven laboratories.

Ceppellini: Not all seven! Maybe two.

Amos: Yes, sure, by some. Now one minute. I need to amplify what Trnka said. I spoke this morning about WHO collaborating laboratories and we were proposing that it would be easy to set up 3 or 4 in the U.S. and, you know, other ones in Europe, and possibly one in Australia. It's pretty obvious where they should be. I think we'll agree on that before we leave here. And they'll be the ones that will be responsible for the identification of new specificities. And verification that a new serum has the specificity of one of the existing reagents. Roy?

Walford: This is the WHO Nomenclature Committee and I think all we should do is decide on nomenclature, and not start setting up laboratories.

Bodmer: But we have to think into the future, Roy. Someone is going to come up with a serum and say they've tested out their panel and it's none of the things we know about, therefore, a new antigen. And it's going to come back to this committee and we are going to have to define it. We will want to see the data. We will want to have criteria on the basis of which we can make such a decision. We're doing it now from the Workshops. But if we don't have any Workshops, we don't have any basis.

Ceppellini: No, but we can list already a number of specificities where all the laboratories have some good sera within their hands, which have been controlled through Workshops. The same laboratories may undertake the job of controlling a new specificity, a new serum. I mean, they are working, they are progressing, they are trying to enrich their own experience looking for sera, typing the panels. When they have something worthwhile they send to the serum bank a report of what is going on. Otherwise, there will be a direct exchange of sera, as we did already.

Trnka: There is International reference Laboratory – WHO World reference Laboratory.

Walford: For red cells?

Trnka: It's in London, and there are international standards but to get an international standard recognized by the International Standard Organization Committee – you know this requires six years sometimes, because you have to collect the material – then send it for international try, and then to submit it to the committee for approval.

Walford: Well, the point I'm making is, investigators have made tremendous progress for 20 or 30 years using anti-D, a very well-defined antigen, without having it standardized or referred to or anything. Why do we suddenly need all this in the white cell field when the red cell field has grown up very well almost without standardization?

Amos: I take real exception to what you say about the modeling of cells on the red cell type. I think it's scandalous that anti-D wasn't defined years ago. And I don't think we should model ourselves on this.

Trnka: You know, I do agree with you, because in red cells you have 3 groups of nomenclature, and the people would rather die than to accept another one.

Walford: Well, we're the Nomenclature Committee. I'm for deciding the nomenclature, but not for setting up a huge apparatus of certification. Someone was just talking about 7 laboratories.

Trnka: Me. Me. It was me. But I would like to say: look, you will have someone in Yugoslavia. How would you expect that this man, getting a paper from WHO, will see there is now a leukocyte typing going on. Whom he will write to, his government, to WHO? WHO will give it to us and we will choose someone to recommend. Here you are giving to the scientific community some names, some laboratories, some bases, and everybody can choose what he wants. we are only facilitating collaboration. Once we will decide these 6 or 7 collaborative laboratories, we will even escape WHO because I will be able to put them in an attractive state.

Bodmer: I think I can see one of Roy's worries and I'm sure that all of us would feel the same. It's how many millions of sera will those poor devils who are specified in that WHO leaflet suddenly have to start comparing. This is something that has to be faced.

Walford: Is there anybody here who would like to be a participant in one of these laboratories?

Ceppellini: I want to.

Bodmer: I think we'd all do it.

Walford: That's what I mean. We are doing it.

Ceppellini: We've been doing it for years. There is an advantage to be gained, because you keep your panel up to date. On the other side, what I tried to solve in Italy is that if a new laboratory wants to get into leukocytes, he must send somebody to work with me for some time, and he will type his own panel in my laboratory.

Bodmer: I don't mind doing this if, first of all, it's clear that someone doesn't send you a serum unless they've done enough with it to make it likely that it's useful, and secondly, if they do it somewhat on the condition that if you find it's useful maybe they'll give you a standard. This is the obvious reciprocal relationship.

Amos: Let's discuss the specificities we've talked about, the first three – 1, 2, and 3. What, for example, would you suggest as a reference standard for 1?

Ceppellini: If a new laboratory wants to type, we give, we or WHO or NIH, or one of us, give a serum which is a good anti-LA1 or 2, and this new laboratory types his own panel. But, what to do more than that? Why should we again now put a standard for LA1. There's no need for that.

Walford: I would make the following proposal. If you want to standardize at WHO or some other place; have a 100 cell panel available. Anybody can send in sera but they have to say what it probably is. It's anti-Mac or anti-LA3 or something. And it'll be typed against the 100 cell panel, and they send back that it is or it is not, and how closely it conforms with the typed paned. And that's all. Thus you avoid everybody sending in a whole batch of sera.

Dausset: A new specificity! So you don't get the new specificity this way!

Amos: It gets back new and then it gets distributed to other laboratories to see whether they find it new and then it automatically gets a new designation.

Batchelor: I have sympathy for what Roy was saying right at the beginning. This committee is terminology of antigens and not trying to erect some sort of organization for typing everybody's sera. The point is that if somebody thinks they've got something new, then you should put the onus of responsibility showing it's something new upon them. And that means, if they really are serious they could go to any convenient panel that has been typed. If they're near Torino then they approach Dr. Ceppellini. So - and what we could do is define the minimum number of cells that we would find acceptable before accepting that it is a genuine new reagent. But it would be up to everyone to prove it for themselves.

Rood: I agree with everything that's been said before, and especially with Richard, and I think this is an extremely good idea. I think a large panel cells could be frozen and made available for such tests to laboratories who think they've got something new. If we want to prevent that we get into the same mess as we have been in the past, then we should discuss points like this.

Amos: Excuse me. I would have thought something approaching 200 would be desirable.

Walford: I think that's larger than you need for most things. It depends on the frequency of reaction.

Shulman: You're not talking about frequency of reactions. You're talking about whether it matches with your known positives and negatives and the Chi Square.

Walford: That depends on the frequency of reactions.

Amos: Well then, and it's got to be absorbed, and you know, who's going to do this?

Batchelor: Well, he has got to do it himself first, he or she, the person who believes that they have got a new specificity.

Walford: The way to establish a new specificity is to write good papers in the literature.

Ceppellini: That's right. I agree completely.

Terasaki: I wonder, you know, I would agree with Richard, but I think we're talking about a higher court of judgement. That is, after you have decided that it's something new, how do you know that it's different from somebody else's sera, and so it seems to me that if we all got together, and had serum exchanges at specified intervals.

Ceppellini: In fact, there should be some kind of obligation that we exchange, that when we think we have a new specificity, we provide the laboratories with the sera.

Bodmer: I must say that I'm beginning to change my mind in agreement with Richard and Jon, that this collaboration can be a sort of unwritten thing, and that it exists simply by nature of the people on this committee whoever is publishing in the field. And perhaps it's going too far to designate it formally.

Amos: All right. Now suddenly, we've agreed that we're going to accept these specificities we've discussed as being defined on the basis of the Torino Workshop data and on the strength of exchanges, and published data. Is that unanimous that we are accepting specificities what were called LA1LA2LA3, 4d 7c, 7b?

Dausset: The 5 was also defined well by Ceppellini and by Kissmeyer-Nielsen.

Rood: May I make a move to make 7c and 7d 7 and 8, whatever you want to do.

Amos: Discuss. 7c is 7.0, 7d is 8.0.

Ceppellini: Do you mind we change 7d to 7 and 8 to 7c?

Amos: Agreed and the definition of LA1LA2LA37c7d as defined in Leiden, Turin and other exchanges. Now, are these additional specificities to be discussed?

Dausset: The 5, the 4. The 5 is much better than the 4c.

Amos: Well then, do you want to include the 4?

Ceppellini: 4 I think is 11.

Dausset: 4 is 11, yes, but it is not so good as the 5, and maybe the F1 of Shulman which is 14 for us. We have many examples, many complement fixation.

Bodmer: But is it an HLA antigen.

Dausset: Oh yes, 14 is in the 4a series.

Ceppellini: And we can propose LA4.

Amos: Wait a minute. I'm writing this down: LA4, Lc-11.

Payne: What's the name of Lc-11?

Walford: It's Hunt B, which is LA4.

Bodmer: We have LA4 too. I would think that there has to be more comparison to designate LA4.

Amos: I'm making a list. That's all among them. This doesn't imply anything about making a list with equivalents. What's the Torino equivalent of the 8?

Ceppellini: Torino 5.

Amos: All right. Now let's go on. What else?

Payne: 4c.

Amos: All right.

Owen: That's the same.

Amos: As what?

Owen: Dausset 5.

Batchelor: Now that we're getting to the antigen or specificities which we're less certain about, don't you think we ought to define just a few of the criteria?

Amos: No, let me get my list completed, and then I'm going to attack it. Now, Paul at one time suggested that one of the things that we might do was to give provisional classification. You see, we're all agreed on this first set. But included in these second ones there are some that we're pretty hopeful about, and some that the data is mostly in one laboratory, only one panel apparently. So I think this data is more at a provisional level. How could we designate that?

Trnka: I would suggest that we put a big table into our memorandum and on that table have the sure specificity and the other specificities which are on the way to be confirmed. We can designate a provisional number for this future specificity Because if something happened and this specificity will be dropped, nothing has happened. We can reserve the number for something else or drop the number. We will have in the future difficulties if we won't do that, because someone will come with a specificity and say I have the right, the number and so and so. But having a provisional number, we can say this number is already reserved. You can start from, let us say, 21.

Bodmer: Yes, I think then it's a terrific mistake to designate something unless we're absolutely sure.

Amos: But it would be helpful to people to have various tables. I don't see any reason why we, if we did agree that 45 was a useful initial designation for 4a, couldn't state it.

Walford: You're giving a provisional nomenclature.

Amos: But to anything.

Walford: Well that's even better.

Dausset: I agree with Walter on this point: only for the very well-defined sera.

Bodmer: Listen, 5 is very well-defined, isn't it?

Payne: Where are we?

Amos: 5 is well-defined.

Ceppellini: Excuse me, I would decide about the number of this 5 sera, or we go down into criteria.

Amos: All right, O.K.

Payne: Let's go into criteria.

Amos: Let's go into criteria. Shall we take an example? Shall we take Dausset 5?

Dausset: It's a bad example.

Walford: There should be two sera available on an international basis both of which are monospecific which give identical reactions and it should be two laboratories.

Trnka: But then you have to designate the laboratory.

Amos: Why do you need two sera.

Ceppellini: Because if there is one it's enough to have the original name.

Trnka: Yes, I think that the 2 sera is at least

Bodmer: There's a big logical distinction between having one and having two.

Trnka: Nobody can agree.

Walford: I'll give you a specific example. Lc-20 has been studied a lot. It's monospecific. It seems to be well-defined specificity according to me. But nobody else found it yet.

Amos: Lc-20. Yes, we have it.

Dausset: It's beautiful.

Walford: On the other hand with regard now to Lc-11 or Torino 12 or Dausset 16, which is kind of like LA4, this is a specificity that has now been found in three separate laboratories. It's quite well-defined.

Amos: Suppose two sera are almost the same and not quite the same. Then what do you do? Do you give them a name or not?

Ceppellini: It's very simple. We don't give them a designation today but just exchange sera and in one month's time we shall know.

Amos: No, you're confusing two things. One is the criteria we're going to put, and you're talking about fulfilling the criteria. What are we going to accept as a new specificity? Roy said it has to be two antisera that give similar or almost similar results.

Batchelor: Well, I would like to break up the criteria into testing at least two panels in different laboratories which have already been tested with the accredited sera, so that we know whether it's different. That would be one limb of your criteria. The second is that there would be published somewhere data showing that the serum is monospecific, and we can define what we accept as being monospecific. Thirdly, we ought to put down a few genetic criteria, test a few families to show that it's linked with HLA antigens.

Amos: Well, the first one is almost axiomatic, two or more laboratories having a known panel. I don't think there's any dispute about this.

Batchelor: But shouldn't we say how many cells?

Payne: Yes, you've got to have at least, say, 10 positive cells.

Amos: And the sera must be closely related by virtue of a given r value, and that value is yet to be determined.

Ceppellini: Let us say that the name before the point – the integer – defines a specificity. Closely related specificities by a given state of r have the same integer plus plus designation after it.

Amos: Don't forget that what I am writing here is not the final language.

Dausset: Would it not be possible to get a preview, so we could look at it?

Amos: Yes. Well, it'll only, it may take one or more sera to define a specificity. Each serum must be strong and give high reproducibility on two panels containing approximately 100 cells or more. Any discrepancies to be resolved by repeat testing and by absorption where necessary! Each sera will be functionally monospecific as judged by absorption with at least 30 cells. If the frequency of reactivity is greater than 15% then at least 15 positive cells must be used.

Bodmer: I'd like to put in a strong vote of dissent against the figure 30.

Amos: All right. Let's bring this back as a matter of detail. The same criteria will apply to the definition of new specificities. In this case it will be the responsibility of the investigator to perform the absorptions in the preliminary testing. Final testing will be performed by at least 2 laboratories as defined above. The new specificity must be controlled by new HLA determinants as shown by studies in at least 3 families comprising at least 10 informative children. So we have not much time.

Bodmer: Why can't we take copies of this back with us and subunit our corrections within a certain length of time?

Trnka: I think that we can speak only about the language in the corrections. Because once you make a bigger correction then I have to correspond to everyone and to ask their approval for the change. And that is really impractical. The best would be to have one evening during that next week for approval of a text. Then once I will have the text I would think that only minute changes or correction of errors can be made. Otherwise I will never get through.

Amos: Because we want to have somebody read the test at the Transplantation Society meeting.

Trnka: Yes.

Walford: I don't think it's that critical that we have such absolute agreement on this, because we're not going to be able to enforce how many absorptions everybody is going to do or not. The only thing we really want to try to enforce is the nomenclature.

Ceppellini: Not completely.

Amos: It's a bit more than that. We would like to set a high standard, I think. Isn't that the general feeling?

Bodmer: It's anyhow a suggestion.

Dausset: It's not a suggestion. If they want to have something recognized they must follow it.

Ceppellini: That's right. Otherwise they are on their own.

Amos: Now is there anything of substance I've left out?

Trnka: You have left out that in the future the letter or the number should be reserved in front of the number, before the decimal point for subloci.

Bodmer: A letter can be placed in front of the integer decimal system to define those that belong to the same sublocus.

Dausset: Symbol! You shouldn't say letter or number. Say "symbol."

Trnka: It means we will fight again for different kinds of symbols.

Bodmer: Always got to have something to discuss.

Amos: All right, so then, certain specificities can be named on the basis of presently available evidence – and we will say what they are – and letters may be placed in front of the symbols to designate subloci.

Bodmer: Do you want to define what an r-value is? Do you think this is going to be clear enough for all people?

Someone: Yes.

Ceppellini: We must at least establish a number of reference laboratories.

Trnka: Not reference but collaborative laboratory.

Ceppellini: Collaborative laboratory?

Trnka: These are the laboratories that are willing to collaborate and I accepted that as good.

Bodmer: I think that establishes too much of an "in" group.

Amos: Gentlemen! We don't have very much time.

Ceppellini: That's an important point.

Amos: You are underestimating the people who will write these various subsections. If there are things that don't get incorporated but have been discussed, then we can do it. But don't prejudge. And my conclusion is that we won't get this report written until we've finally reached agreement on the specificities that we've made. We have to devote a quarter of an hour to tidying up the question of the specificities themselves.

Dausset: I realize that it is quite unrealistic to change the LA nomenclature; I agree that LA nomenclature will remain. But you must also realize that in France and also in Europe and in Holland the Mac is used also. So, I would like and I'm sure you will agree to accept the proposal of Jon which was to make an alternative of my specificity too.

Amos: O.K. Let's go on. Do we call LA4 4?

Rood: And 7, maybe 7c.

Bodmer: And 8, 7d.

Ceppellini: Maybe leave 10 numbers for the LAs. You have to take it up to 9. I don't know.

Walford: We're getting into a problem now with the positive correlation because, you know, like 7d has a positive correlation with Mac.

Rood: I'm not accusing Walford this morning with his neat orderly mind for making difficulties. But I think it is not important to have a logical or orderly nomenclature. Because as our knowledge will increase what sounds now logical will be utter stupidity. What is important is that we have the numbers which are most important in clinical work and as much as possible in agreement with previous nomenclature. That is far more important than whether or not it is logical or their allelism or something else which will be upset anyhow.

Dausset: Please. If you are willing to put afterwards some symbol in front, I agree completely.

Amos: Do you want now to define the r values or some more specificities?

Rood: Why don't we clean up the mess of 7d?

Amos: All right.

Bodmer: I would like to suggest an alternative. Make c as 7.0 and d as 75.

Amos: That implies too much.

Bodmer: Why?

Trnka: Well you just put it outside of the whole nomenclature because once you make such a difference between 1, 2, 3 and then 75, everybody will look and say "What a difference"!

Ceppellini: 7 is 7c and 8 is 7d for me. That's my opinion.

Amos: Well, we already did vote on this. We'll revote on it.

Ceppellini: In this case let us have logic not to make implications but to try to respect as much as possible the present terminology. Now here we have 7c and 7d. There are no special relationships between the two. Now it just happened that, for instance, Dr. Dausset is using 8 instead of 7. Therefore that's a very good reason for having 7 for 7c and 8 for 7b. But it's not particularly logical.

Dausset: O.K., and afterwards a genetical implication.

Rood: That has nothing to do with it. It is only as much as possible confirm its old uses.

Amos: The chair would like to cooperate. We've got a proposal here that HLA7 should be the old 7c and HLA8 should be the old 7d. Could we now take a vote and see whether we have agreement on this?

Walford: Well, I think Ruggero and I would rather have 7d be 7 because we're already both using 7 for 7d. It's not critical.

Amos: O.K. You're voting against.

Shulman: I would like to instead of vote ask a question? How many people's minds would you have to change about anything other than those around this table or if you named something one way instead of another.

Someone: Quite a few.

Rood: We are working on the moment in Europe with 20 to 30 different transplantation teams. Say that each team consists of four or five people who have our results. And then we have people we work together with Africa, Israel and South America.

Amos: Let me please, let me vote total members just to see if there's any substantial change.

Bodmer: Well, I put this forward as a suggestion and I don't really feel strongly one way or the other. I think that having a 75 is not necessarily bad.

Amos: I believe that the 75 is completely out of line with our agreement before. It implies a positive relationship.

Rood: Our nomenclature does not imply anything. It should not imply logic or a genetic relationship, but only be as little confusing for the outside world as possible.

Ceppellini: Let us take 8 because a number of people are using 8. 8 is close to 7.

Amos: All right. I'll pass. Rose?

Payne: I'll accept it.

Batchelor: I like 7, 8 and I don't mind which way around you call them; d can be 7 or 8 as far as I'm concerned.

Amos: Fine. Paul?

Terasaki: I think that we still have to look at this problem as a whole. You know that the way it stands now there's going to be three empty spots. And if we decide that 4c is going to be one of them then I think we're going to be left with 5 not numbered. In which case, people are going to ask why is it that one number you left out.

Ceppellini: But the philosophy is to change the minimum as possible.

During the night various subcommittees wrote parts of a provisional report, and the evening and the morning were the

SECOND DAY

Bodmer: We should call ourselves the HLA committee.

Shulman: Do you think some HLA antigens might be restricted to certain tissues or cells?

Ceppellini: There is an antigen, which is expressly fibroblast, and not a lymphocyte, but is genetically linked with something belonging to HLA.

Owen: It says on page one..."the present report concerns nomenclature of antigens within the system that currently are well recognized, and proposes to identify the naming of these specificities.

Amos: All right now. All we need to do is add this sentence, "It does not relate to other antigenic systems such as those involving granulocytes or platelets, and other lymphoid and tissue antigens which need to be considered separately.

Dausset: An example is good, very good there. To put in an example is certainly good.

Amos: Such as Na-1? Or what do you want?

Owen: Couldn't you get this by just saying "concerns only nomenclature of antigens within the system"?

Dausset: I think it is good to have an example because then it is information even for the people who are not involved. Because someone who is not involved doesn't know what you are talking about once you speak of whole systems. There are many, many systems, for example Communist system.

Terasaki: Suppose we reconsider Kissmeyer-Nielson for that matter.

Amos: Well, we'll ask him.

Ceppellini: Kissmeyer I think we can put him in because we can ask tomorrow morning.

Bodmer: At the end why don't we simply say that "the laboratories are those selected by the members of the committee and other qualified laboratories whose consent will be obtained." And let me talk about this more obviously, because that's what this list is, really: it's just a list of us.

Ceppellini: That will be useful. I could go to Rome and say "Look! Look"!

Bodmer: That's for you Italians. I can't go to the NIH and do that, at least I don't think so. Maybe I can.

Batchelor: When we've gone round and asked everybody, if I remember correctly, Dr. Trnka is a member of the commission, but who else?

Amos: That's the problem. I've been trying to avoid it.

Dausset: No, no. I think it's better to have it in the report but I would like to have from you here the necessary guidance.

Walford: May I make an offer?

Trnka: Yes.

Walford: Van Loghem or Englefriet.

Trnka: Should we ask Morris?

Amos: Morris because of his strategic location.

Bodmer: There are other people in Australia.

Bodmer: Well, I don't know that anyone has a complete outfit in Australia.

Dausset: Let us add only the ones, let's put only Kissmeyer-Nielsen and Englefriet.

Bodmer: Those people who are "in," and no one else.

Payne: Man! (laughs)

Rood: Can I make a point here, Mr. Chairman?

Amos: Yes sir.

Rood: I think for us this table of equivalents may be quite clear, but as this will be published and will be used by the people who have been receiving our reports in the past, I think it's confusing the way is stands here. For instance I would very much prefer we had been using LA1 and LA3, etc.

Bodmer: So you'd like to include them in?

Rood: Yes.

Bodmer: Well, include them in.

Rood: We have been using in the past LA1, LA3 and Oz-5...

Bodmer: But that's fine!

Rood: This table will be used. We are working together with twenty and thirty centers. Paul is working together with fifty of them.

Amos: Okay, so you add LA1 and the others.

Batchelor: Isn't that 8a? You have 8c there.

Rood: 8a it is.

Amos: LA3, and 5 is also 5.

Bodmer: Just 5?

Amos: No, Da-5.

Bodmer: Right. Five.....five.....five!

Payne: Walter, if van Rood uses LA3 and so on, shouldn't we be using 7d?

Ceppellini: Sure.

Payne: Put that down in that slot! And would you put 4c in with Dausset 5?

Amos: Now, what have you got? What have you got in there?

Payne: 7b.

Dausset: 7b under 8, yes?

Payne: Yes. Right.

Dausset: And under 5? You have nothing?

Bodmer: Nothing.

Amos: All right. Ruggero, have you got anything?

Ceppellini: Torino 20 for 7, for the new 7.

Walford: Now look, gentlemen, I went around and asked everyone in Torino, personally, about your equivalences and so forth. That is how this chart was first constructed, and published in a number of places. Now we're changing it a little bit, and the equivalences become different. Six months from now we'll change it again, and they'll become different again, and nobody will know what's up. Let's not make things equivalent unless they really are. For example, as I remember, on Torino F-1 was equivalent to Dausset 14. Now we're going to make it equivalent to Dausset 5 and publish that and maybe six months from now it'll go back to Dausset 14. That will certainly confuse everybody.

Bodmer: Look, if you're not sure, it's better to leave it out. I mean, you don't gain anything by putting it in.

Amos: The criteria for identity of reagents we've discussed but we didn't include in our new list. So we need now a pragraph for the acceptability of a similar apecificity.

Batchelor: I'm sorry to be awkward about this table, but I was just talking to Paul. I have included underneath the column headed "Batchelor" only those antigens which give sufficiently close correlation on the Torino workshop or the live workshop, to be in the "Identical" column. Paul's things don't all fit into the "Identical" column. Now if that is going to be the situation, we're having different standards.

Ceppellini: The important thing is that now Paul recognizes that standard for his groups.

Allen: Actually, if you want to define it so, this is only what Paul calls this specificity, whether he has a serum of that sort or not.

Bodmer: I think that also by implication we can say that, come to this problem about accepting a given serum as one of the specificities, and you can say it must be tested on a given number, and we'll state how many positive and negative cells must be present and a given minimal level of disagreement.

Ceppellini: For the last column, under Kissmeyer, we put LA1, LA2, and LA3.

Bodmer: Doesn't he also have a 7b?

Dausset: I think that he has 7c, I don't know if he has a 7b.

Payne: I don't think people should send out cells, really. It's the sort of thing where we'll just be working all the time to send out type cells.

Amos: Would you accept something like this? "For two sera to be considered to define the same specificity, different criteria were suggested" This is after the bit on the definition of the different antigens. "The sera should give almost identical results when tested on two panels totalling at least a hundred cells. There should be no discrepancies on repeated testing or following absorption."

Walford: That's not adequate. I've worked that all out statistically in the Torino paper and it depends on the frequency.

Amos: All right, so we've got "at least a hundred cells containing" – we discussed this yesterday – "at least 10 positive or 10 negative cells." Right?

Walford: Then I think it would be correct.

Amos: Down to Table "A." Batchelor's HLA-5 and Dausset 5 are identical. Anything else?

Ceppellini: You know, let us assume that we made a mistake in that Torino 5 and Dausset 5 are not the same. What would become of 5.1?

Payne: Thank you.

Dausset: The decimal point!

Bodmer: Ah...no, no, no!

Dausset: Let's move on the the next point, and then come back to this decimal point.

Shulman: Could you say something why different specificities should be separated by a decimal point?

Dausset: No one would understand what you mean.

Ceppellini: You should be thankful.

Bodmer: It's an Americanism. You know, it's very American.

Amos: It's very American. No English speaking person would understand what you're saying if you use periods.

Ceppellini: And I tell you, no Latin-speaking, I don't know about German. I don't understand what is meant by decimal points.

Dausset: Why don't we make it a comma?

Bodmer: No, no, no, no, no.

Batchelor: A decimal point, I think...

Owen: In the following sentence I would suggest that the word "generally" be moved forward in the sentence, "specificity designated by the same number will generally have a high, positive correlation."

Amos: No, They'll always have a high correlation coefficient.

Owen: Not neccessarily. Not if it is an inclusion.

Bodmer: No, I'm not sure I'd use that because I think you want them to have a high positive correlation which generally is statement point 9. You don't "generally want a high positive correlation."

Amos: That implies that sometimes you allow a low positive correlation.

Owen: Yeah, and it seems to me leaving it where it is then, does restrict your freedom to use something that is not so highly correlated, but is quite definitely part of the character you are trying to describe.

Ceppellini: What about A-1 and A-2? I think you should have one point one, and one point two.

Amos: I don't understand this sentence, "The effectiveness limit on the three combination of fractions is set by resolution of human pedigree studies."

Owen: I think that might be left out.

Bodmer: No, I think that's very important, because that's really what sets the limit to what you can do in human populations.

Dausset: Notation system! In the third line, the word "variant." I don't think "variant" is a good word. It implies a chemical difference. It is not a genetical term or a zoological term.

Shulman: The first complete sentence, and the second complete sentence on page 3.

Bodmer: "Other factors with a high positive correlation."

Dausset: Yes, yes.

Shulman: All right, let me try it on the chairman. Mr. Chairman?

Amos: Yes.

Shulman: The first complete sentence on page 3 and the second sentence are both devoted to the frequency of recombination as well, as a complete sentence on page 2. I think that is a great deal of emphasis on recombination.

Amos: What does that paragraph have to do with the asterisk?

Rood: Oh, yes, but actually you have to be careful because sometimes colons are used colloquially.

Ceppellini: I move we give an example of special forms.

Dausset: You know, in many languages a colon is the same as a decimal.

Trnka: Comma, comma, yes, comma!

Amos: Delete the second period after HLA2.

Ceppellini: Not a second point! Otherwise we are to be confused.

Bodmer: That's a punctuation point. We don't need it.

Rood: I'm terribly sorry, Mr. Charimen, but I have to go. My English is not that good.

Amos: We would all love to join you, Jon. Now then, could we say here – this is page 3 again – that sentence, the first notational sentence, the first notational system, "Prime effect is indicated by number following a point, e.g., "HLA-2.1."

Ceppellini: HLA-2.0 for the original, for the first specificity and HLA-2.1 for variant, or an associated specificity.

Bodmer: All right.

Ceppellini: HLA-2.0 or just HLA-2?

Dausset: No, that's good, saying point 0 because...

Bodmer: I would say not. I would say HLA-2 for the additional factor.

Owen: It can't be left out entirely because what it means is that we have to assign these things to a single system. It we were working with microorganisms, where you can do biochemical analysis, you wouldn't have any of these problems. So that's extremely important.

Ceppellini: To gauge leukocytes like an elephant is damned anatomical.

Owen: Yeah, but that's a common fact. You're just putting facts in there.

Bodmer: What's a common fact?

Owen: This 1% limit.

Ceppellini: You have a point. We are not here to give a lecture in genetics.

Owen: That's right.

Ceppellini: We just say arbitrarily for convenience 1%. If you don't want an explanation, we can just state it arbitrarily.

Trnka: There will be no change?

Amos: No.

(Dr. Amos now reads the final report of the first meeting of the WHO Leukocyte Nomenclature Committee)

CURRICULUM VITAE (abbreviated)

Roy L. Walford

PERSONAL

Professor of Pathology, UCLA School of Medicine (joined UCLA faculty in 1954)

Born:	San Diego, California, 29 June, 1924

EDUCATION AND TRAINING

Pre-medical, California Institute of Technology; Medical, University of Chicago (1948)
Internship, Gorgas Hospital, Panama; Residency, Veterans Hospital, Los Angeles

MILITARY SERVICE

1952-54	U.S. Air Force (Korean War)

SCHOLARSHIPS, FELLOWSHIPS, AND HONORS RECEIVED

1942	Freshman Scholarship, California Institute of Technology
1960-61	Fulbright Research Scholar (for study in Germany, laboratory of Otto Westphal)
1967-68	Commonwealth Fund Fellowship (for study in France, Laboratory of Jean Dausset)
1980	Phil Levine Award, American Society of Clinical Pathology
1980	American Aging Association Research Award
1980	Kleemeier Award, Gerontological Society
1982	Henderson Award, American Geriatrics Society
1982	Senator Alan Cranston Award, FIBER
1985	Erna Yaffe Commemorative Lecture, Fall River, Mass
1990	Visiting Professor, University of Arizona School of Medicine

MISCELLANEOUS ACTIVITIES

1969-83	Member of WHO Leukocyte Nomenclature Committee
1969-84	Expert Advisor to WHO in Immunology
1969-	Counselor, International Histocompatibility Workshops
1978-80	National Academy of Science Committee on Aging
1979	Chairman, National Institute on Aging Task Force on Immunology
1981	Senatorial Delegate, White House Conference on Aging
1985-	Space Biospheres Review Committee

BOOKS WRITTEN

Walford, R.L.: Leukocyte Antigens and Antibodies. New York, Grune and Stratton, Inc. 1960.

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SCIENTIFIC ARTICLES

Over 300 articles devoted largely to HLA and to the experimental biology of aging

The Early History of HLA

Flemming Kissmeyer-Nielsen and Arne Svejgaard

Address: Department of Clinical Immunology
The Tissue Typing Laboratory
Blood Bank and Blood Grouping Laboratory
University Hospital of Aarhus
DK-8200 Aarhus
Denmark

Flemming Kissmeyer-Nielsen

Arne Svejgaard

Scanticon 1975

Jean Dausset

Flemming Kissmeyer

Queen Dronning Margrethe II of Denmark

THE EARLY HISTORY OF HLA

FLEMMING KISSMEYER-NIELSEN, Aarhus AND ARNE SVEJGAARD, Copenhagen

A few points should be made immediatley about the following text. First, it was written rather hastily for reasons we shall not go into. Second, we have taken Paul Terasaki's invitation to give a personal view of the early history of HLA quite literally: we have biased ourselves strongly towards the results generated in Denmark and have not in any way attempted to review the very important results created by others (which does not mean that we do not acknowledge and admire them). Thirdly, we consider the early history of HLA to end in 1975. Finally, although only one of us (FK) is a real "old-timer" in terms of HLA and thus the one invited by Terasaki, we decided to write this text jointly because we worked so closely and well together in this field.

The senior author (FK) graduated in medicine from the University of Copenhagen in 1947 and was trained in clinical medicine and serology in hosptials in Aarhus and one year at the State Serum Institute in Copenhagen, and built up a modern blood bank and blood-grouping laboratory in the University Hospital of Aarhus. After having served the University and the hospitals of Aarhus for 42 years, FK retired in 1989. The junior author (AS) graduated in Medicine from the University of Aarhus in 1964, was trained in clinical medicine at the University Hospital of Aarhus, and in clinical immunology at the department of the senior author from 1965 to 1971.

None of us has been sincerely involved in the very early history of HLA. However, the elder of us (FK) interestingly followed the field closely and introduced the leukoagglutination technique and the complement-fixation technique with platelets in the laboratory in Aarhus.

The microdroplet assay of the human lymphocytoxins was first introduced by Terasaki and McClelland in 1964 in relation to the 1st International Workshop on Histocompatibility Testing, organized by Bernard Amos in Washington D.C. and Duke University. It was also used by Terasaki in the 2nd Workshop, organized by Jon van Rood in Leiden in 1965. This technique was so elegant that one of us (FK) in 1965 decided to study it in further detail, not only in Terasaki's laboratory, but also in that of Roy Walford. A visit was also paid to the laboratory of Rose Payne, who had previously described a number of leukocyte antigens using leukoagglutination. She generously provided us with her previous, highly specific antibodies, including both published and unpublished specificities.

Upon the return to Aarhus, Rose Payne's antibodies were used to establish a local panel of well-typed donors, the most precious instrument to have in a tissue typing laboratory.

On the basis of the experiences in Terasaki's, Walford's, and Payne's laboratories, FK developed together with our excellent technician, the late Karen E. ("Lisbeth") Kjerbye, a robust modification of the lymphocytotoxic microtechnique (1), which became subsequently known as the K-N technique and was used in all Scandinavian tissue typing laboratories until recently. With this technique, we screened pregnancy sera using the panel typed by Rose Payne's sera and succeeded within a remarkably short time to collect a set of lymphocytotoxic antibodies giving very reproducible results. Most of these antibodies detected well-known antigens, but some new antigens. By means of these antibodies and another set of complement-fixing platelet antibodies, we wre prepared to participate in the 3rd International Histocompatibility Workshop in Torino in 1967.

However, before then, one of us (FK) made the group's first significant contribution to the field of transplantation by the demonstration in 1966 together with other members of the transplantation team at the Universtiy Hospital of Aarhus (2) that preexisting lymphocytoxic antibodies against donor anitgens can cause hyperacute rejection of a transplanted kidney. It is of interest that the Lancet first rejected this paper because the reviewer believed so much in the contemporary dogma from experimental animal studies that transplantation immunity is cellullar, not humoral.

The 3rd Workshop, excellently organized by Ruggero Ceppellini in 1967 used, as a main research tool, blood from rather large Italian families living in the neighborhood of Torino. The participating groups brought their antibodies to Torino and tested blinded samples from the families. This gave the organizers an opportunity to check the typing results in relation to the segregation of the antigens. We used our K-N technique for microlympocytotoxicity typing (FK) and the complement-fixation technique with platelets (AS). During the first meeting of the Workshop, it became clear that the organizers had forgotten that we needed a few extra millilitres of blood for the isolation of platelets. When Ruggero realized that, he hesitated for a few seconds and then shouted: "if not blood, no Kissmeyer!". This was not exactly encouraging, but we nevertheless managed to get the blood needed. Ruggero later told us that the next day when he saw that our results of investigating the blinded samples followed the HLA haplotypes precisely, ordered that we should have priority in terms of blood.

The major result of this Workshop and the triumph of Ceppellini was, of course, the demonstration that virtually all human antigens detected by lymphocytotoxic alloantibodies are controlled by one genetic system: HLA. Ceppellini and co-workers (3) concluded that there are at least two mutational sites within this region, but most investigators believed that the contemporarily known HLA antigens were controlled by genes at several, if not many linked loci, in parallel with the contemporary - erroneous - concept of the H2 system in mice.

Our personal benefits from participating in this Workshop were, among others, the confirmation of the reliability of the K-N technique and the observation that many HLA antigens can also be detected on platelets by means of alloantisera in the complement-fixation technique. Accordingly, by using both techniques, we obtained extra accuracy of our HLA typing.

The following year, we demonstrated (4), mutually independently of Dausset and Colombani (5), that some HLA sera are crossreacting, a phenomenon well-known from ABO blood grouping serology, but surprisingly at that time never seriously considered within HLA or H2-serology. This observation of crossreacting HLA antibodies had two immediate consequences: first, it provided a satisfying explanation of the previously cryptic phenomena: ANAP and CYNAP described by van Rood. Second, and most important, it formed one of the pillars for our subsequent simplified model for the genetics of the HLA system. The other pillar was our observation that many of the less frequent, "narrow" HLA antigens were completely "included" in one or more "broader" antigens, some of which in turn were completely included in the two classical 4a and 4b, now HLA-Bw4 and Bw6 antigens. The posititve (and negative) associations between various HLA antigens was well-known, but the accuracy of our typing allowed us to detect the complete inclusions, which, together with the existence of cross-reactivity, was the basis for postulating together with the late Mogens Hauge, professor in genetics at the University of Odense, with whom we had started a fruitful collaboration, that all contemporily known HLA antigens fitted completely into two segregant series: the LA and the FOUR series, now HLA-A and B (6). Both population and preliminary family studies were in complete agreement with the assumption that each of these two series behave as if controlled by alleles. During a visit to Dausset's laboratory in the spring of 1968, one of us (FK) told about these findings, but they said that their data did not fit our hypothesis. However, a few months later at the Transplantation Congress in New York, their data did also fit.

The validity of these two series was supported by our observation together with Lilian Staub Nielsen, the University Hospital of Copenhagen, of a family in which a recombination had occured between HLA-A and B (7).

We realized that if the HLA-A and B series were the whole truth of the HLA system, HLA-AB identical unrelated individuals should be mutually non-responsive in MLC, and in the autumn of 1968, we started a collaboration with Soren Freiesleben Sorensen, who had introduced the MLC technique at the State University Hospital in Copenhagen. We selected pairs of HLA-AB unrelated individuals from our file of HLA-typed individuals in Aarhus and sent blood to Copenhagen for MLC investigations, which to our disappointment showed stimulation in most cases (8). This could either mean that still unknown splits of the HLA-A and/or B series were responsible, or that still unknown series were involved. The latter, of course, turned out to be true.

In 1968, we also started a collaboration with Erik Thorsby, the University Hospital of Oslo, who was producing excellent alloantibodies by planned immunization of volunteers which allowed definition of new HLA antigens, among others the so-called "FJH" antigen (9), now HLA-B27, one of the most clinically relevant antigens at the present time. Both groups continued to find new specificities although it was sometimes frustrating for one of us (AS) that it was much harder to find complement-fixing platelet antibodies than lymphocytotoxic ones. Together with Erik, we collected further family data, which allowed us in a joint analysis (10) of a large sample to strongly support the hypothesis of the two segregant series, HLA and B.

The existence of these series was, of course, established beyond doubt during the 4th International Histocompatibility Workshop organized by Terasaki in 1970.

However, already in 1969, Lena Sandberg of the University of Gothenburg had found an antiserum detecting a peculiar antigen, "AJ" (now HLA-Cw1), not fitting in either with the HLA-A or the HLA-B series. Lena kindly sent samples to Aarhus and Oslo, and our combined studies showed that AJ was partially included in several of the narrow HLA-B specificities. Was it a new series or was it peculiar splits of HLA-B antigens? Both possibilities could be true at the time, but still we dared to suggest the first possibility during the Workshop Meeting in 1970 (11). The royality of the HLA-C series was, of course, not established until the subsequent capping experiments of Solheim et al, (12) and the discovery by Staub Nielsen of addittional anitgens (new HLA-Cw2, Cw3, and Cw4), which we noted fitted nicely into this series (13).

Before the Workshop, one of us (FK) founded "Scandiatransplant" after the model of "Eurotransplant", introduced earlier by Jon van Rood. Scandiatransplant has now functioned since 1969 as a cooperation between all transplant groups in the Nordic countries and until now, about 10,700 necrokidney transplantations have been performed in Scandinavia. Over the years many colleagues from Scandinavia and other countries have been in Aarhus to learn HLA typing.

In 1969, one of us (AS) demonstrated that HLA antibodies may act synergistically (14), which may cause sera containing several different antibodies to give erroneous typing results, and completed his thesis with the somewhat misleading title: "Iso-antigenic systems of human blood platelets" (15), - its main emphasis was on the genetics of the HLA system. The following year, the other (FK) together with Thorsby, published an extensive review on the HLA system (16).

In 1971, our ways parted when one of us (AS) was appointed Head of the Tissue Typing Laboratory at the University Hospital of Copenhagen, but we continued our collaborations although necessarily of a lesser intensity. For example, in an extensive collaboration with all Tissue Typing Laboratories in Scandinavia, we were able by means of complete ascertainment of all typed informative families, to give the first accurate estimate of the crossing-over frequency between the HLA-A and B loci (17). We found it to be 0.8%, which is very close to subsequent results obtained by others.

In Aarhus, Lars U. Lamm now joined the group and discovered the linkage between the HLA system and the PGM_3 locus (18) and was subsequently able to assign the HLA complex to chromosome 6 in a family with a pericentric inversion (19). Population studies were continued and in connection with the 5th International Histocompatibility Workshop, organized by Jean Dausset, extended to include Eskimoes from Greenland (20). Our results clearly showed the homogeneity of the "pure" Eskimoes from the East Coast of the country in contrast to the Caucasoid admitxure of those from the more inhabitable West Coast.

For odd reasons, there was one area in which we did not collaborate: attempts to establish MLC (HLA-D) typing. As a result, Fritz Jorgensen in Aarhus (21) and Bo Dupont (22) in Copenhagen simultaneously solved the problem by means of homozygous typing cells in 1973. Fritz had his results published very rapidly, while Bo went to present his at a meeting in St. Paul de Vence (organized by Fritz Bach) to learn that the groups in Munich (23) and Leiden (24) had also solved the probelm. Moreover, the latter group also provided hints as to how serological MLC typing might be done, and one of us (AS) still remembers the stir in the room during the meeting, when Ad van Leuven dared to suggest (25) that the MLC determinats were on B-lymphocytes rather than T-lympocytes as was generally believed at that time.

The Copenhagen group got interested in HLA and disease associations early in the seventies. We were concerned about the statistical probelms and recieved help form Mogens Hauge and Walter Bodmer (26). In 1973, one of us (AS) together with Lars Ryder established the "HLA and Disease Registry" in Copenhagen (27) and started collecting published and unpublished data on this topic, which has been a laborious, but fruitful job.

As soon as MLC-typing had been established, Casper Jersild, who had previously confirmed the association between HLA and multiple sclerosis (28) and Bo Dupont together with Torben Fog immediately applied the HLA-D typing technique to a sample of patients with multiple sclerosis and found that the HLA association in that disease is probably stronger with HLA-Dw2 than with HLA-B7 (29) - the first demonstration of an association between a class II antigen and a disease.

In 1973, Singal and Blajchman (30) showed that the insulin-depndent form of diabetes mellitus (IDDM), but not the non-insulin-dependent form, is associated with HLA-B15, which was a break-through both in terms of the acceptance of these two disorders as separate genetic entities and in relation to understanding the etiology of IDDM, which was at that time not generally accepted as an autoimmune disease. However, these investigators honestly stated in their paper that they had observed some triplets involving B15, and accordingly, the results were not generally accepted. Consequnetly, we embarked together with Lars Ryder, Per Platz, Jorn Nerup, and Jens Lyngsoe on a similar study and were able not only to confirm the HLA-B15 association, but also to discover the B8 association (31). By subsequent analysis of these data, one of us (AS) noted that HLA-B8/15 heterozygotes have the highest risk of IDDM (32). Mogens Thomsen performed HLA-D typing of IDDM patients and provided strong evidence that the association is stronger with HLA-Dw3 and w4 rather than with HLA-B8 and B15, respectively (33). Now, it is, of course, known that HLA-B8/15 heterozygosity "effect" is secondary to such an effect between DR3 and DR4 or between DQw2 and DQw8, - or between other combinations of these determinants. This interaction still remains unexplained, but it would appear to hide a clue to the etiology of IDDM. We also found evidence that other autoimmune disorders are pirmarily associated with class II determinants (33).

In a collaborative study (34) between Aarhus and Copenhagen including the dermatologists Hugh Zachariae and the wife of one of us (AS), we were able to demonstrate that psoriasis vulgaris and pustular psoriasis are separate entities from an immunogenetic point of view.

It seems logical to end this survey with a note on the 6th International Histocompatiblity Workshop (35), which was organized by us in collaboration with Erik Thorsby. The Workshop established that HLA-D typing by means of homozygous typing cells is indeed feasible using a data reduction method developed by Lars Ryder (36). On the basis of these results, the WHO Nomenclature Committee named the first six HLA-D determinants. The first five HLA-C antigens were also named. The Conference took place in the high summer of 1975 at "Scanticon" in Aarhus, and the efficient secretariat was run by Elly Andersen, who has helped us so well over all the years (including the typing of this manuscript). Other angels may have been around the Conference, where the Aarhus group presented data on a family named Christensen, which was abbreviated "Family Christ" on the slide. This apparent relation to higher powers was used by some participants to explain the exceptionally brilliant weather which characterized both weeks of the meeting. This good weather comes to our minds when thinking of all the good collaboration we have had and all the joyful hours we have spent with our fellow HLA workers all over the world.

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THE GENETICS OF THE HLA-SYSTEM

F. KISSMEYER-NIELSEN, Aarhus

The HLA-system is a genetic system of immense complexity, which is, like e.g. the ABO and Rhesus systems determined by allelic genes (i.e. genes which exist in two or more alternative forms and determine the same character in different individuals) on one of the 23 pairs of human chromosomes. Today it is known that the genes of the HLA-system are located on chromosome No. 6. By a genetic system is now understood two or more regions (loci), each with two or more genes located close to one another on the same pair of chromosomes. It should be remembered, however, that for decades the term "system" has been used about blood group systems though they have only one locus belonging to them, and it will probably be difficult to do away with this well-established terminology.

Several Genetic Loci

About 30 years ago only one HLA-locus was known. This locus had an ever increasing number of allelic genes, each coding for corresponding antigens on the surface of all nucleated cells in the organism. A single individual could have a maximum of two different genes belonging to this series, viz. one on either of the two chromosomes, and there might be only one allele coding for only one antigen (homozygosity).

Later research has established the existence of more loci belonging to the HLA-system. Today four - or perhaps five - loci (A, B, C, D/DR) of greater or less importance for transplantation are known. Besides it is known that other polymorphic genetic systems are determined by allelic genes on chromosome pair No. 6, more or less closely linked to the HLA-system.

Research at the Tissue-Typing Laboratory

In connection with the very short outline of the genetics of the HLA-system given above, it should be mentioned that the Tissue-Typing Laboratory of the Municipal Hospital of Aarhus has played an important part in the exploration of genetical details.

A team comprised of F. Kissmeyer, A. Svejgaard, and M. Hauge was the first to provide clear evidence of the existence of the two HLA-loci now called A and B (1). F. Kissmeyer and his collaborators further described the first certain case of crossing-over within the HLA-system (2). It should also be mentioned that Lars U. Lamm, in cooperation with the Tissue-Typing Laboratory, was the first to demonstrate linkage [=hereditary characters located on the same chromosome (No. 6)] between the HLA-system and another genetic marker (PGM_3) in his dissertation from 1971.

Finally it should be mentioned that A. Svejgaard and F. Kissmeyer were the first to provide clear evidence of cross-reactive HLA-antigens (3).

Loci of Importance for Transplantation

The HLA-loci proper, A, B, C, D, and DR, have had various names through the years but now follow the alphabet according to their time of recognition.

These loci are the ones which are important for transplantations. To each of the A, B, C, D, and DR loci of the HLA-system belongs a varying number of allelic genes. The final number is yet unknown, unidentified genes (and corresponding antigens) belonging to each of the loci. These unknown genes, termed "blanks", are simply pending the discovery of reagents to reveal the antigens they code for.

At present (1980) the following number of allelic genes (and antigens) belonging to the HLA-system have been described in Denmark.

A-series	17
B-series	27
C-series	8
D-series	12
DR-series	10

Though at most two genes from each series (one on each of the two chromosomes in pair No. 6) can be present in an individual, obviously all genes of all series are capable of innumerable combinations. E.g. just the 17 and 27 different genes belonging to the two oldest HLA-loci, A and B, respectively, may combine to 459 different HLA-haplotypes (the HLA-genes on one of the chromosomes). The 459 different HLA-A,B haplotypes may be combined in 105,570 different HLA-genotypes (when the genes of both chromosomes are included). The 17 A-genes may be combined in 154 A-phenotypes, and the 27 B-genes in 379 different B-phenotypes. These A- and B-phenotypes may be combined to 58,366 different HLA-phenotypes or tissue types. It will appear that this is quite an extraordinary degree of complexity or polymorphism.

However, the different HLA-A and -B combinations are not equally frequent, and the most frequent A and B combination is present in only about 1 percent of the White population. If genes belonging to the C, D, and the possible DR loci are included in the calculations, the number of possible combinations rises from tens of thousands to millions. The practical result is that each unrelated individual is "unique" - "no" other individual in existence has exactly the same HLA-type if genes belonging to all the loci known today are taken into consideration. During the Torino Workshop in 1967, it was impossible to find two different Bw4 and Bw6 antibodies giving exactly the same reaction pattern.

Bw4,w6

It is another question whether some of the antigens (and the correspondent genes) may need some revision. The substantial number of inclusions of narrower antigens in broader ones was seen already in our Nature article from 1968 (1). A shorter presentation of some of these typical results appears in Table 1.

Linkage Disequilibrium

It should be pointed out that the different HLA-genes in the population do not combine to the different haplotypes at ran-

Table 1. Inclusions within the 4 series - Bw4,w6

ANTISERA					
I	II	+/+	+/-	-/+	-/-
Bw4	Bw44	76	68	0	134
Bw4	B5	34	104	0	127
Bw4	B13	14	107	0	110
Bw6	B7	90	62	0	122
Bw6	B40	12	43	0	43

dom -they are unevenly distributed, and some haplotypes occur much more, some less frequently than expected. This has not been explained, but it might be due to genetic selection and e.g. related to the connection between the HLA-system and certain diseases (v. below).

Naturally, the complexity described above makes it difficult to find unrelated individuals who are even reasonably alike as regards the total HLA-type.

THE HLA-SYSTEM AND TRANSPLANTATION

Heredity

The problem is different in the case of members of the same family. As already mentioned, the HLA-system is hereditary, and the hereditary characters are located on one pair of chromosomes as linked genes. The result is that it is comparatively easy to find donors in the patient's family with tissue types similar to his own. The manner of succession in the HLA system is shown in Figure 1.

In this figure, the father's HLA chromosomes are designated A and B and the mother's C and D. During the formation of the sperm cells and egg cells, the chromosome pairs are split, as is well known, and half of the mother's egg cells will carry the C chromosome, the other half the D chromosome. When the fertilization taken place, there are only four possible combinations: AC, AD, BC and BD, which means that in a family of brothers and sisters there will be a 25 per cent chance that two individuals are HLA-identical - and this is the ideal situation for transplantation, provided, of course, that there is ABO-compatibility. The father or mother will also be relatively good donors for their children, because the parents will always have at least one HLA chromosome in common with all their children. Consequently, any incompatibility in the case of transfer of an organ from the father or mother to one of their children will be attributable to only one pair of chromosomes - not to two as in the unrelated situation - and there will be a relatively good chance of acceptable compatibility.

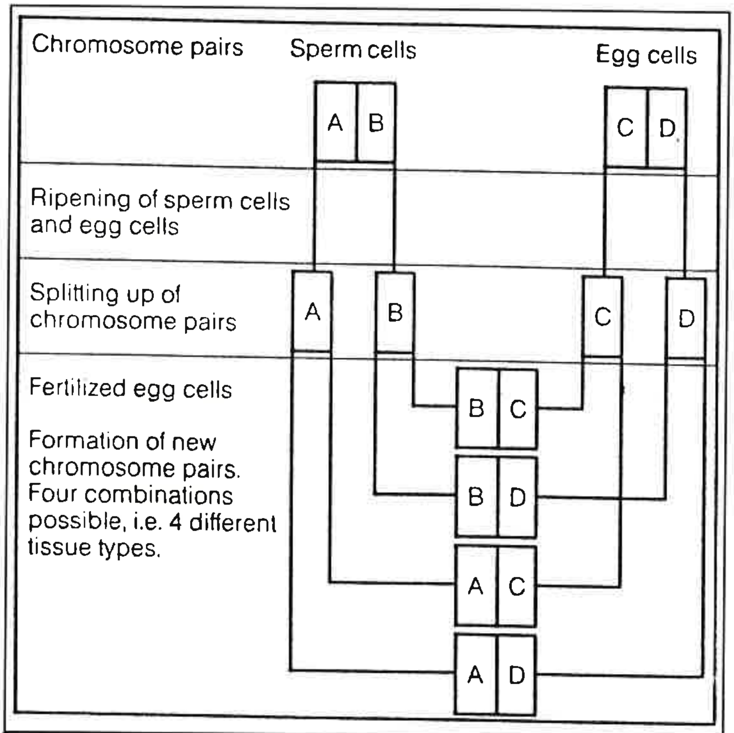
Chromosome pairs
Sperm cells
Egg cells
A
B
C
D
Ripening of sperm cells and egg cells
Splitting up of chromosome pairs
A
B
C
D
Fertilized egg cells
Formation of new chromosome pairs. Four combinations possible, i.e. 4 different tissue types.
B C
B D
A C
A D

Figure 1. The mode of inheritance of the HLA-system. The genetic information is located on chromosome No. 6, called A and B in the male and C and D in the female. (Of course these names have nothing to do with the A-D designations used earlier).

Kidney Exchange Programmes

The difficulties of finding unrelated individuals of the same HLA-type have led to the establishment of international programmes for the exchange of kidneys from deceased persons. The possibility of taking into consideration the HLA-types of donor and recipient is increased by gathering recipients from several countries in a pool so that there is a wide choice when a donor possibility arises (kidneys from a deceased person, necro-kidneys).

Among these projects is Scandiatransplant, organized with F. Kissmeyer as prime mover on the same model as Eurotransplant which was established by van Rood in 1968 (comprising the Benelux countries and West Germany). Scandiatransplant was established in 1969 and comprises all the potential recipients of the transplantation centers in Denmark, Finland, Norway, Sweden, northern Germany (Hamburg), and Iceland. The tissue-typing laboratories involved (a total of 9) are kept informed of changes in the recipient pool and handle the complicated communication connected with it. Suitable recipients were originally traced by means of a special punch-card system. The need for rationalization has grown with the size and rate of activity of the pool, and central EDP registration and peripheral EDP contact over the telephone network via teletype terminals have now been introduced.

During the period 1969-1979, about 5,000 necro-kidney transplantations were performed within the Scandiatransplant programme (an average of about one per day), and the recipient pool seems to have stabilized at about 600.

Identification of Suitable Donors/ Recipients

When planning the transplantation of an organ, it is essential to take into consideration the known strong antigens belonging to the ABO- and HLA-systems and check the following points:

- ABO-compatibility between recipient and donor (i.e. antibody active against donor ABO-antigen must not be present in the recipient).
- The HLA-identity must be the best possible.

The importance of the HLA-system in the selection of transplantation donors is proven by the results of transplantations from related donors, particularly the almost 100 percent of successes achieved by using HLA-identical brothers or sisters as donors. The ordinary related situation also shows a clear correlation between the degree of incompatibility (the number of HLA-antigens which are present in the donor and absent in the recipient) and the results achieved.

The HLA-A,B-identical necro-kidneys also give better results than kidneys from one or two HLA-A,B-incompatible individuals, but the picture is less clear than in the related situation. However, analyses made at the time when the immunological factors must be supposed to play the greatest role - viz. about ten to twelve days after the transplantation, there is, according to quite recent analyses made by Ole Fjeldborg on the Aarhus material, a highly significant correlation between loss of the transplanted organ and the number of HLA-A and -B incompatibilities.

The Survival of the Transplant and HLA-Compatibility

The correlation between the survival of the transplant and HLA-A and -B incompatibilities appears from an analysis of the Scandiatransplant material made on the 1st January, 1980, by Lars U. Lamm. The material comprises a total of 1,695 transplantations using kidneys from deceased persons, and an observation period of at least 160 weeks. The clear (and highly significant) correlation between

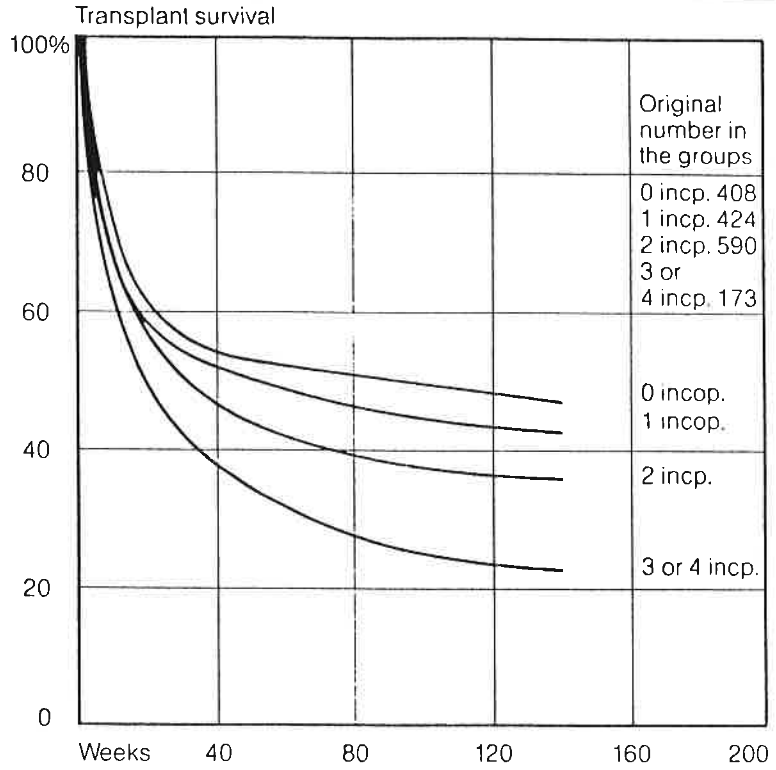

Figure 2. The correlation between transplant survival and HLA-A,B incompatibility (Scandiatransplant material, January 1980).

the degree of compatibility for the HLA-A and -B antigens and the transplant survival appears in Figure 2.

The Importance of the DR-Antigens

During recent years it has turned out that compatibility for the recently serologically recognized DR-antigens seems to play a greater role than the compatibility for the HLA-A and -B antigens mentioned above.

This appears from i.a. and analysis which Ole Fjeldborg, our transplantation surgeon, has now made of the Aarhus material. This material comprises as yet the comparatively small number of 52 transplantations, but there is a significant correlation between DR-compatibility and transplant survival (p=0.003), as shown in Figure 3.

It may be added that there are corroborative results from w.g. Oslo, Stockholm, and Rigshospitalet (the University Hospital) in Copenhagen. This inspires optimism with regard to the achievement of substantially better results from future necro-kidney transplantations - though it should be pointed out that there are still many technical and practical problems attached to DR-type selection.

Innumerable other curves showing the importance of selection for transplantation according to HLA-types exist, and it should be emphasized that this method of selection is of importance not only for the fate of the transplanted kidney but also for the patient's survival.

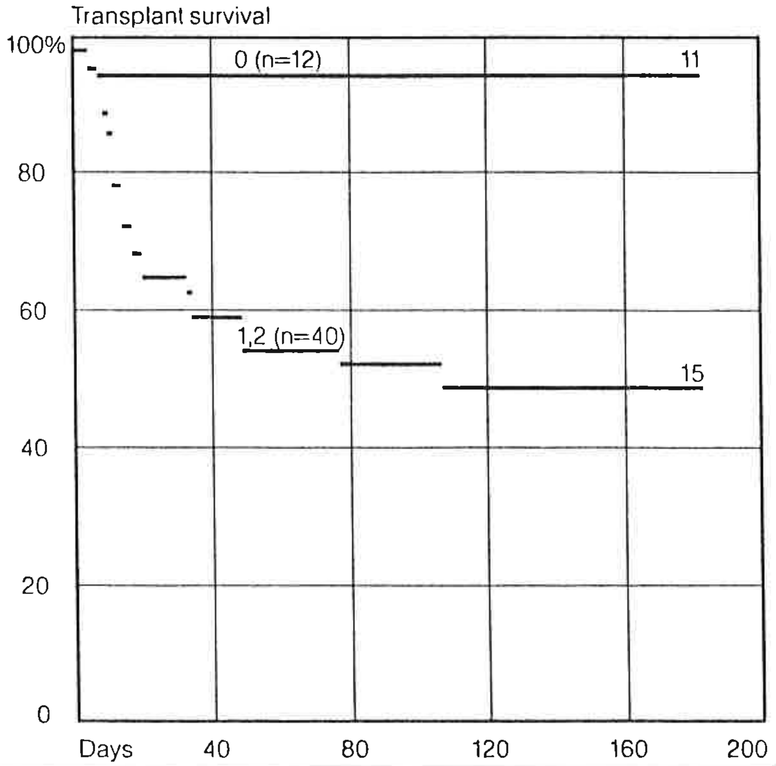

Figure 3. The correlation between transplant survival and DR incompatibility (Aarhus material, November 1980).

Cellular Immunity

Though Figure 2 shows a clear influence of matching from HLA-A and -B antigens, the results are not as good as those achieved when HLA-identical sisters and brothers are used.

Part of the explanation of this difference between the related and unrelated situations is undoubtedly the fact that strong TP-antigens are also determined by MLC locus, now termed D locus. This locus seems to determine the intensity of the cellular immunity elicited in vitro (and presumably in vivo, too) in the so-called "Mixed Lymphocyte Culture". The D locus is closely linked to (is placed on the same chromosome pair as) the other loci of the HLA-system. The close linkage between HLA-loci has the effect that by making sure that there is HLA-A and -B identity between brothers and sisters, one will almost always also have made sure that there is MLC-identity, while this is not so in the case of transplantation between unrelated individuals.

The MLC-Test

Until recently, the only way of identifying the MLC information was the MLC-test. This test is made by mixing living "responder" (or recipient) cells with "stimulator" (or donor) cells treated with mitomycin (a substance which prevents cell division) or with large X-ray doses. After five or six days' culture of this mixture any sensitization of responder cells can be registered either visually by blasttransformation (increasing percentage of immature responder cells), or better still by measuring increased DNA synthesis, usually registered by the ability of the cell culture to incorporate radioactively marked thymidine, which takes part in the DNA synthesis.

MLC locus seems to be of decisive importance for the development of the cellular immunity which is attached to T-lymphocytes and in its turn, decides the fate of the transplant, but as the LC-test takes five or six days, it will seldom be possible to apply this test previous to a necro-kidney transplantation.

The cellular immunity established by sensitization can be demonstrated by the "killer" effect of sensitized T-lymphocytes against i.a. other living lymphocytes from the sensitizing donor. This can be shown by the so-called CML-test (Cell-Mediated Lympholysis). The stimulating donor lymphocytes are marked with radioactive Cr^{51}, and the "killer' effect to the sensitized recipient lymphocytes can be recognized by the liberation of Cr^{51} after four to six hours' culture between the two lymphocyte populations involved. It should be pointed out that the demonstrated "killer" effects seem to have an extremely pronounced HLA-A,B specificity, i.e. it is demonstrable not only against lymphocytes from the sensitizing donor but also against lymphocytes from other individuals of the same HLA-type.

It seems, then, that the establishment of cellular immunity is determined by MLC locus while the killer effect is HLA-A,B-oriented, though it has recently been proved that there are special determinants which are independent of HLA-A,B (and -C) but can also be targets for the killer effect. Such loci, too, are linked to the HLA-system.

At the Tissue-Typing laboratory in Aarhus, Tom Kristensen and Niels Grunnet have given special attention to these problems.

DR-Antigens

Within the last couple of years it has turned out that by applying a serological technique (lymphocytotoxicity) it is probably possible to determine the determinants belonging to the D locus. These are the so-called DR-antigens. They are termed DR (=D Related) because it is not known yet whether they are identical or very closely associated with the D determinants.

DR-typing by serological technology can be completed within four or five hours, and this opens up the possibility of selecting donors for transplantations according to DR-types. The findings so far seem to show that this has improved the results substantially so that in the case of compatibility for HLA-A,B and -DR, the results are approaching those achieved from transplantation from HLA-identical brothers and sisters, as appears from Figure 3.

Humoral Immunity

Humoral immunity, which is also important for the fate of the transplant, seems to be exclusively HLA-specific. This appears e.g. from the fact that the HLA-A,B similarity between donor and recipient seems to be of greater importance when HLA-antibodies are present in the recipients at the time of transplantation. The harmful effect of the antibodies on the transplant seems particularly to be conditioned by the activasion of the complement mentioned in connection with tissue-typing.

Acute Rejection

The importance of HLA-antibodies also manifests itself by the hyperacute rejection of a kidney transplant (minutes or a few hours after the transplantation), which is seen if a kidney is transplanted to a recipient in whom HLA-antibodies active against donor HLA-antigens are present.

In collaboration with the kidney transplantation team of the Municipal Hospi-

Table 2. Hyperacute rejection of kidney allografts

K.-N. et al., Lancet Sept. 24, 662, 1966

Principal serological data before transplantation:

Case No.	Pregnancies	Blood- & Plasma-transfusions	Leucocyte agglutinins titre	Thrombocyte antibodies (C'fix)	Reactions obtained with donor antigens:		
					Leucocytes	Thrombocytes	Kidney extract
14	5	52	1/512	+	++++	-	+++
17	3	34	1/512	++	++++	++	++

tal of Aarhus, F. Kissmeyer was the first to prove that the normal circulating antibodies might cause acute rejection of the transplant (4) (Table 2).

This complication is so dreaded that a direct compatibility test where the recipient is examined with regard to donor lymphocytes is always attempted before a transplantation - and the transplantation is only carried out if the result of the test is negative.

Prevention of Presensitization against Transplantation Antigens

Both cellular and humoral immunity are decisive for the fate of the transplant, and in an attempt to reduce the possibilities of either of these developing prior to the operation, the following measures are taken:

- As few blood transfusions as possible are given.
- The number of antigens in the blood is reduced by using blood with a low content of leucocytes (and, implicitly, of trombocytes).
- If possible, blood of the same HLA-type as the recipient's own is used - which is very rarely feasible owing to the complexity of the HLA-system.
- In the case of retransplantation, transplants of the same incompatibility as earlier transplants are avoided as far as possible.

Quite recently, some doubt has arisen as to the importance of blood transfusions in connection with kidney transplantations. Material from a good many operations has shown a lower rate of transplant survival in patients who had not had blood transfusions prior to operation, but the problem cannot be regarded as having been cleared up so far.

A Type in Danish Eskimos from Greenland

HL-A typing of 110 Danish Eskimos has shown a marked difference in the frequencies of some HL-A antigens as compared to Danish Caucasians.

The most striking finding was a high frequency of HL-A9, Ba*(W28), HL-A5, BB(W10), and FJH(W27). There was a low frequency of HL-A1, Li(W19), HL-A7, HL-A8, haplotypes in Eskimo matings, and the importance of this in relation to the high frequency of trophoblastic lesions among Eskimos is discussed (Tables 3 and 4).

Table 3. *The frequencies of the different LA and FOUR antigens in Eskimos and Danes*

Antigen	Eskimos Positive No.	Eskimos Positive %	Danes Positive No.	Danes Positive %	χ^2	p
LA series	*N = 110*		*N = 334*			
HL-A1	4	3.6	93	27.8	28.40	< 0.001
– 2	40	36.4	180	53.9	10.17	< 0.01
– 3	12	10.9	93	27.8	13.14	< 0.001
– 9	87	79.1	70	21.0	103.67	< 0.001
– 10	2	1.8	28	8.4	5.66	< 0.025
– 11	7	6.4	35	10.5	1.64	> 0.05
Ba*(28)	21	19.1	27	8.1	10.40	< 0.01
Li($>$19)	1	0.9	65	19.5	22.50	< 0.001
				$\chi^2_8 =$	195.58	< 0.001
FOUR series	*N = 110*		*N = 289*			
HL-A5	24	21.8	22	7.6	15.76	< 0.001
– 7	9	8.2	78	27.0	16.53	< 0.001
– 8	2	1.8	64	22.1	23.85	< 0.001
– 12	4	3.6	69	23.9	21.83	< 0.001
– 13	1	0.9	13	4.5		> 0.05 [a]
R*(18)	15	13.6	38	13.1	0.02	> 0.05
BB(10)	39	35.5	64	22.1	7.37	< 0.01
FJH(27)	35	31.8	28	9.7	29.34	< 0.001
LND(15)	26	23.6	51	17.6	1.84	> 0.05
SL($>$17)	5	4.5	31	10.7	3.71	> 0.05
AA(22)	1	0.9	7	2.4		> 0.05 [a]
JA(?)	13	11.8	0	0.0		< 0.001 [a]
				$\chi^2_9 =$	120.25	0.001

N = number of individuals tested. The numbers in parentheses after the antigens listed in the first column refer to the workshop numbers used during the 4th workshop. HL-A1, 2, 3, etc. refer to the WHO nomenclature.

[a] Fischer's exact test was used for these calculations.

Table 4. The gene frequencies for the LA and FOUR genes calculated by means of the gene-counting method (see Methods)

	ESKIMOS		DANES		
	Total	East Greenland		t	p
LA-series					
HL-A1	0.0182	n.o.	0.1557	8.01	< 0.001
2	0.2040	0.029	0.3211	3.33	< 0.001
3	0.0545	n.o.	0.1497	4.50	< 0.001
9	0.5711	0.6647	0.1091	10.31	< 0.001
10	0.0091	n.o.	0.0423	3.29	< 0.001
11	0.0318	n.o.	0.0524	1.39	> 0.05
Ba*(28)	0.1041	0.157	0.0427	2.72	< 0.01
Li(>19)	0.0045	n.o.	0.1059	7.77	< 0.001
'0'	0.0027	0.1487	0.0211		
				$\Sigma t^2 = \chi^2_8 = 282.32$	< 0.001
FOUR series					
HL-A5	0.1110	0.147	0.0392	3.08	< 0.01
7	0.0422	n.o.	0.1466	5.08	< 0.001
8	0.0091	n.o.	0.1174	7.11	< 0.001
12	0.0188	n.o.	0.1253	6.28	< 0.001
13	0.0046	n.o.	0.0229		
R*(18)	0.0716	n.o.	0.0725	0.04	> 0.05
BB(10)	0.1997	0.396	0.1179	2.58	< 0.05
FJH(27)	0.1826	0.059	0.0500	4.58	< 0.001
LND(15)	0.1276	0.311	0.0948	1.24	> 0.05
SL(>17)	0.0239	n.o.	0.0586	2.42	< 0.05
AA(22)	0.0049	n.o.	0.0145		
JA(?)	0.0838	0.061	0.0000		
'0'	0.1202	0.026	0.1403	0.36	> 0.05
				$\Sigma t^2 = \chi^2_{10} = 160.44$	< 0.001

n.o. = not observed. '0' indicates the frequency of genes which may exist, but which we are unable to detect, most probably because of lack of antisera. The t-values were calculated for the "total" Eskimos compared with the Danes. The Danish gene frequencies are from Thorsby et al. (1970).

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CURRICULUM VITAE

Flemming Kissmeyer-Nielsen

PERSONAL

Born: January 3, 1921

EDUCATION

1947	Graduated in Medicine from the University of Copenhagen
1950	Gold Price Medal in Medicine, the University of Aarhus
1954	Ph.D. in Medicine, the University of Aarhus

POSITIONS

1947-70	Clinical and serological training at the University Hospital of Aarhus (medical, surgical, paediatric, pathological-anatomic and X-ray department) and the State Serum Institute of Copenhagen
1968	Specialist in blood group serology (in 1971 the name of this specialty was changed to *Clinical Immunology*)
1953-56	Lecturer in pathological anatomy, the University of Aarhus
1956-71	Lecturer in blood group serology, the University of Aarhus
1957-89	Director of the Blood Bank and Tissue Typing Laboratory, University Hospital of Aarhus (in 1971 the name of the department was changed to *Department of Clinical Immunology*)
1971-89	Professor in Clinical Immunology, the University of Aarhus

OTHER ACTIVITIES

1971-89	Editor for TISSUE ANTIGENS, Munksgaard, Copenhagen

PREVIOUS SCIENTIFIC PRIZES

1968	Rigmor and Carl Holst-Knudsen's prize
1974	Hede Nielsen's prize
1977	The Rosenkjaer's prize
1980	The Novo prize
1982	Karl Landsteiner Vorlesung. Deutsche Gesellschaft für Bluttransfusion und Immunhämatologie, Würzburg
1982	The Anders Jahre's Medical prize
1987	August Krogh's prize

PUBLICATIONS

Total number : 300

The Kidney Transplantation Team

in

Aarhus, Denmark

O. Fjeldborg, Kidney Surgeon — Steen Olsen, Pathologist — F. Kissmeyer & V. Posborg Petersen, Immunologist, Nephrologist

Kissmeyer pre workshop, 1975

"This Sixth Workshop is too much work"

Flemming with Ekkehard Albert

Sixth Workshop, Aarhus

Roy Walford　Max Baur　Ekkehard Albert　Flemming Kissmeyer

Roy	Max	Ekkehard	Flemming
Walford	Baur	Albert	Kissmeyer

Rose	Walter	Julia	G.M.T.	Hans
Payne	Bodmer	Bodmer	Schreuder	Grosse-Wilde

Sixth Workshop

Z. Trnka | Jon van Rood | Jean Dausset | Roy Walford

Post workshop at the country house
Flemming Kissmeyer, Jean Dausset

Random Memories of HLA

J. Richard Batchelor

Address: Department of Immunology
Royal Postgraduate Medical School
Hammersmith Hospital
Du Cane Road
London W12 ONN
England

Richard Batchelor ca. 1962

Kuwait, 1990

RANDOM MEMORIES OF HLA

J. RICHARD BATCHELOR

After some rumination, it seems best to begin with a disclaimer. Memories, like our T cell repertoires, are the result of selective processes, and although these are largely unconscious, they nearly always impart a bias towards showing the writer and his opinions in a favourable light. I have always felt that this is the true meaning of Henry Ford's aphorism – History is bunk. It might therefore be more correct to refer to what follows as "impressions" rather than a "memoire"; no notes have been kept by me on the events of the early days of the studies on HLA. Nevertheless, within the limits of a fallible memory, these are some of my recollections.

In 1958, just before completing two years of obligatory national service in the Royal Army Medical Corps, I visited the Pathology Department at Guy's Hospital, my clinical alma mater. George Payling Wright, the head of the department at the time, had always impressed me with his deep understanding of pathology. He introduced me to Peter Gorer and it was eventually agreed that if the Medical Research Council were prepared to award me a research studentship, I would join Peter Gorer's group in the Pathology Department at Guy's Hospital Medical School. Thus in January of 1959 I began my apprenticeship.

I was full of uncertainty – had this been the right decision? Although pathology had always attracted me since undergraduate days at Cambridge University, Peter Gorer's work seemed so far from the clinical medical scene. It took me the best part of a year to begin to understand the full scope of Peter's perception, that the mouse MHC was potentially an important model for man. No doubt I was slow-witted, but at the time transplantation immunology was not on any syllabus, there was almost no clinical transplantation, and there were very few laboratories in the field. Futhermore, Peter was understandably not given to long conversations with his newest recruit. Teddy Boyse, who had joined Peter's lab a couple of years earlier, was an important educational force for me.

By the time I joined Peter's group, he and his students (notably Bernard Amos) had defined many of the H-2 specificities by haemagglutinating and lymphocytoxic antisera. They had invented the serological methods, applied them to the analysis of half a dozen strains of mice, and demonstrated the multi-gene nature of the H-2 system by finding the first intra-H-2 cross-overs and establishing breeding colonies from them. The technical approach and model for the human homologue was thus displayed.

My first projects were concerned with two other interests of Peter's, tumor-specific transplantation antigens and immunological enhancement of the growth of tumour allografts. I did not therefore become personally involved in the analysis of the H-2 system although I used the same serological techniques. At the time, there was an occasional discussion about investigating human material for a homologue of H-2, but no action was taken.

Peter died unexpectedly in 1961, of lung cancer. Ridiculous though it may be, I was the senior person left as Teddy Boyse had moved to the United States. I had barely two years of research experience and found that setting longer term research goals and advising a Ph.D. student at that stage was not easy. I remain deeply grateful to Peter Medawar and Leslie Brent who did their best to help me in those days. Bernard Amos was also characteristically generous, inviting me to visit his laboratory first in Buffalo, and then later for three months in 1963 at Duke University. During my stay at Duke, I became familiar with the published studies of Jean Dausset, Jon van Rood, and Rose Payne on leukoagglutinating antisera and tried my hand at their methods, in company with Bernard Amos. Neither of us felt confident with the leukoagglutination test. I suggested we should try the lymphocytotoxic technique which worked so well with murine cells, but Bernard had already thought of that and explained that the problem was the lack of a satisfactory complement. He had tried sera from a variety of animal species as a source of complement for use with human alloantisera, but had not identified one. It was only at the end of my three-month sabbatical period that we heard that Roy Walford and his co-workers had a manuscript in press for Science which showed that rabbit serum was an effective source of complement for human alloantisera(1). Recently, when talking to Paul Terasaki, I learned that Paul's group had also studied a variety of complement sources and that Paul had first reported the effectiveness of rabbit complement at the New York Academy of Sciences in February of 1963, but the publication did not appear for another 20 months, in November 1964 (2).

Back in London in early 1964, I decided to join the serious investigation of human histocompatibility antigens. The pioneer renal transplant units were already showing that with azathioprine and steroid therapy, unrelated donor kidney grafts succeeded in some cases but not others. The possibility that we might be able to predict that fate of these grafts was obvious. Frank Ellis, a surgical colleague at Guy's, taught me how to skin graft, and I put up a notice in the Medical School asking for volunteers. In those times, there were no ethical committees. We did give thought to the possible dangers of viral hepatitis transmission, requiring all volunteers to become blood donors, and following the recipients of their blood. None of the recipients of the blood, or the skin grafts developed any infectious complications. The first study included 14 volunteers, most of whom produced useful lymphocytotoxic antibodies after 3 consecutive skin grafts. It was by no means clear at that time whether any of the well established red cell antigen systems were also histocompatibility factors. I was interested to find that although our skin grafted volunteers produced lymphocytotoxic antibodies, none of them developed any haemagglutinins (3). This made it clear that the human homologue of the mouse H-2 system was not one of the previously identified human red cell antigen systems.

Although in the early history of HLA, significant discoveries were made by individual scientists, the most powerful force driving progress in the field was the series of Histocompatibility Workshops, often just called the HLA Workshops. Nowadays, the concept of Workshops, not merely for HLA, is so familiar, that it may be difficult for younger scientists to imagine a time without them. It would be easy to fail to recognize their revolutionary impact. I believe it is fair to claim that the series of HLA Workshops were the first of their kind and have demonstrated just how much can be achieved by this approach.

The first Workshop held in 1964 was due to the initiative of Bernard Amos and Paul Russell. In retrospect, I regard its most important achievement as being the introduction of a practical laboratory session into a conventional conference setting. This opinion of the first Workshop may be a little harsh; I was not at the meeting, having returned to London from Bernard's laboratory at Duke University only a few months earlier. At the time, there was a heated debate going on about what were the most reliable and sensitive methods of detecting alloantibodies against human leucocytes. Jean Dausset had demonstrated the first leucocyte alloantigen, Mac, using sera from patients sensitized by blood transfusions (4). Rose Payne and Rolfs (5) and Jon van Rood et al, (6) had shown that leucocyte antibodies could also be found in the sera of multiparous women. Thus, there was no shortage of sera for analysis, but there was no consensus of opinion as to the best technique for identifying leucocyte alloantibodies. This important technical issue was not resolved at the first Workshop.

In 1965, Jon van Rood and his colleagues held the 2nd HLA Workshop in Leiden. It was a remarkable event, for which Jon and his many helpers deserved (and received) great credit. The principle of doing laboratory work at the Workshop was maintained, but for the first time a proper scientific experiment was planned and executed. Sixteen teams analyzed reactions of their own antisera on the white cells or platelets from a panel of 45 blood donors. I remember being deeply impressed by the organizing ability of the Dutch group. The teams were provided with a large suite of laboratories and basic equipment such as centrifuges and incubators for a week. Each morning a set of blood samples taken from the Leiden panel of volunteer donors awaited us in the laboratories at 8 AM. Each team, using its own favoured methods, tested its own antisera upon the blood samples provided. Every evening, at 6 PM, our results were handed in to be transmitted to the computer department. The atmosphere in the Workshop laboratories was not without its tensions. Competition particularly for the use of centrifuges was acute at certain times in the day. Usually good humour prevailed, but disappointments did lead to some sharp exchanges. I recall Felix Milgrom instructing one of his group at about 4 PM when their complement fixation test had evidently failed, "Vell Kano, ve vill have to start again, von't ve", and then stalking out of the lab. The patient Dr. Kano duly returned to work.

At the end of the week, the computed results became available. It has to be remembered that 1965 was several years before the micro-methods of Paul Terasaki and his group had been fully developed and universally adopted. Virtually all of us were using approximately 10-15 µl of sera per test, and corresponding amounts of target white cells. Before reading cytotoxic tests, supernatant serum and complement was removed by Pasteur pipette from each tube, trypan blue (or eosin-y) added and mixed, and then the cells transferred to a counting chamber for microscopic evaluation. It was all very cumbersome and slow. Probably the amount of data produced at the Leiden Workshop would be regarded as rather small now, but nonetheless the conclusions were both statistically significant and very exciting. They showed that different laboratories were able to identify the same antigens in many instances, that the extent of the polymorphism was large, and that it was imperative to agree upon a standard terminology flexible enough to allow for new findings. It was also evident that the distribution of some antigens in the panel of volunteers was not random, confirming many earlier studies of individual laboratories.

Largely on the basis of significant positive and negative associations of leucocyte antigens in panels of unrelated subjects, Jean Dausset proposed the existence of a single system, which he named Hu-1. I remember being unimpressed by this because I felt that Peter Gorer and his students had already demonstrated the likely pattern by their analysis of the mouse H-2 system. It seemed most improbable that man was going to differ fundamentally from mouse. What was needed, in my view, was evidence demonstrating associated inheritance (in coupling or repulsion) from parent to offspring of all the newly defined polymorphisms.

I had already determined to follow that trail before going to the 2nd HLA Workshop, but before embarking on family studies, had to be sure that the antisera used to identify any given specificity only contained antibodies against that specificity. To this day, I remember the tedium of that exercise, in which Ann Chapman and I absorbed out antisera with graded numbers of leucocytes from between 10 and 20 subjects for each serum, and then tested each absorbed serum again on our panel of white cell donors. Our volunteer donors were equally glad when we decided we knew how many antibodies the sera contained (7). The next task, recruiting and bleeding families, was highly educational for me. As a medical student learning midwifery, I had delivered infants at the homes of poor people living near to Guy's Hospital. But I was unprepared for the difficulties faced by some of the families who generously agreed to volunteer as white cell donors. One family, documented in the publication of our family studies (8), had 13 children. They lived in 3 small rooms and they had a large, fierce, alsatian dog. I had to take blood kneeling on the floor with the dog growling in my ear. The strategy was to bleed the youngest first, and because he did not cry, the remaining children were honour bound also to be brave. I had to bleed them during daylight because the electricity had been cut off for non-payment of the account. I reported the inheritance of the leucocyte antigens we were able to detect in the parents and offspring of 17 families at a symposium on tissue and organ transplantation in London, early 1967 (8). Later that year, the Third Histocompatibility Workshop organised by Ruggero Ceppellini and his colleagues took place in Torino. Eleven families were tested by the 16 teams attending the Workshop and the data confirmed that most of the leucocyte antigens detected at that time were determined by a single genetic system. No recombinants were identified.

Like the 2nd Workshop, the 3rd was an exciting experience. Ruggero had managed to borrow some wonderful new laboratories constructed of marble. Each day we worked at the .bench until approximately 7 PM and were then carried off to dine at a trattoria. A young technician whom I brought to help tasted her first mouthful of Italian red wine, and fell asleep across the table shortly afterwards. It goes without saying that I, not Ruggero, was the one accused of having drugged her. The 3rd Workshop was the last at which it was possible to accommodate all the participants in a single laboratory. This organisation gave the early Workshops their sense of comradeship even though there was also much competition.

There wasn't sufficient time after the 3rd Workshop to discuss all the data in detail; one result was that Ceppellini's suggestion that the HLA system identified so far appeared to form two allelic series polymorphisms provoked a delayed, rather than immediate response. Most of the arguments were aired (rather vociferously) at the next Workshop and eventually it was agreed to name them HLA-A and B. By that time (1970), the increasing number of scientists who were interested in HLA genetics made it impossible to accommodate them together in a single laboratory. Paul Terasaki, the organizer of the 4th Workshop, very logically decided that there was no need for us all to work together using leucocytes from a single panel of donors; instead, we could work anywhere provided a standard set of antisera were used by all. Such s step would have been impossible but for the ingenious micromethods that he and his group developed, which allowed the small amounts of well characterized Workshop sera to be used by everyone. The importance of this contribution was profound. Without it, our knowledge of the HLA system would have accrued much more slowly and in a patchy, geographical manner. Furthermore, our appreciation of the significance of HLA polymorphisms in medicine would have been retarded because of the shortage of properly standardized antisera.

The 5th Workshop was an adventure for everyone. Jean Dausset rightly insisted that we ought to study populations other than easily accessible caucasoids, and so this became a major objective. We were invited to go on expeditions to collect the necessary material. Peter Morris, based in Melbourne, invited me to join him in the New Guinea highlands. He had made arrangements through the Institute of Human Biology at Goroka in the highlands. All was prepared. I flew via the U.S.A. collecting Roy Walford in Los Angeles, and then on to Fiji. There we collected blood samples from a group of Fijians, prepared lymphocyte suspensions and sent them on to Peter at Melbourne. Later I joined Peter in his laboratory to carry out some transplantation experiments with John Fabre and a few weeks afterwards Peter and I flew to New Guinea. The chief of our expedition was Sandy "have a wee dram" McGregor. He had organization skills of a high order, which included finding sources of rare Scottish malt whisky. He was a most generous host. We visited villages by a four wheel drive truck. Sandy would converse with the villagers and arrange for volunteers. During this gathering, which usually attracted most of the villagers, it was an occasional habit that the older women would welcome a male stranger by sliding a hand of greeting up the shorts and gripping the stranger by his manhood. I had been warned by Sandy, and managed to escape this attention. Thus it gave me added pleasure to note Peter Morris's expression when he, ignorant of the custom, received this unusual welcome.

The 6th Workshop was organized by the group led by Fleming Kissmeyer Nielsen, based in Aarhus on the Jutland peninsula (Denmark). My most abiding memory of it was that Fleming had arranged the conference programme so that tennis players could get a game most afternoons. Peter Morris who was my partner in a series of hotly contested doubles matches remarked on returning to the U.K. that it was the first time he had returned from a conference with "tennis elbow". The "Kissmeyer Workshop" established the existence of the HLA-C locus, and some wondered how much more remained to be discovered.

During the mid '70s, H-2 class II polymorphisms were detected on murine B lymphocytes. This obviously prompted the question whether there were human homologues; the early work of Aad van Leeuwen in Jon van Rood's laboratory indicated that such homologues were indeed detectable. The next Workshop, organized by Walter Bodmer (1977) was designed therefore to analyze the human class II polymorphisms. Walter and Julia asked Hilliard Festenstein, Peter Morris and myself to help. The experimental plan was in essence similar to those of previous Workshop's in which a selected set of antisera were tested by all participants in their own laboratories. The difference was that on this occasion the target cells were to be suspensions of purified B cells. Considering the relative inexperience of most labs in preparing B lymphocyte suspensions, and the crude techniques available then, it was a surprise that the Workshop was so successful in defining the DR specificities. In addition to the serological part of the Workshop, Dw typing with Homozygous Typing Cells (HTC) which had been introduced at the previous Workshop was also included in the 7th Workshop. It was fascinating to find the significant correlations between the serologically defined DR specificities and the HTC defined Dw ones.

The next 2 Workshops in 1980 and 1984 resulted in much refining of the definition of both class I and class II polymorphisms. The additional class II loci were defined and some of the class III region genes mapped. But no one doubts that the advent of molecular genetic technology has entirely changed the scene.

In retrospect, the system which so many of us spent a large part of our professional lives investigating has become so familiar to everyone involved in biomedical research that it may appear boring to talk about those earlier days. However I am sure that the participants in the early Workshops certainly did not find HLA dull. They were exciting times.

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SHORT CURRICULUM VITAE

J.R. Batchelor

Richard Batchelor (born 1931) read Natural Sciences for Medicine at Cambridge University in 1949-1952. During his last year he took the Part II of Natural Sciences in Pathology, and this experience strongly attracted him towards the subject of Pathology. Between 1952 and the end of 1955 he did his clinical medical studies at Guy's Hospital, London and then spent 1 year as an intern before being called up into the Royal Army Medical Corps as a National Service Medical Officer. In 1959 after completion of National Service, he joined Peter Gorer's laboratory in the Department of Pathology at Guy's Hospital Medical School. He presented his M.D. thesis in 1965. In 1967, he was invited to become the Director of the McIndoe Memorial Research Unit at the Queen Victoria Hospital, East Grinstead. Here he was able to develop his interests in transplantation immunity which included HLA matching for renal, skin, and corneal allografts and immunological enhancement of rat kidney grafts. He was appointed Honorary Research Professor in the Royal College of Surgeons (England). In 1979, he moved to a personal chair in the Department of Immunology, Royal Postgraduate Medical School, Hammersmith Hospital, London in order to be able to study HLA disease associations more easily. In 1982 he was appointed Head of the Department of Immunology.

At Vald' Aosta near Turin after Third Workshop, 1967

During sabbatical with Peter Morris *John Fabre* *Richard Batchelor*

Fifth Workshop preparations, 1972 Fiji Island

Volunteer blood donors, Fiji Island tobacco plantation

The T Lymphocyte Perspective of HLA: A Personal Reflection

Fritz H. Bach

Address: Immunobiology Research Center
Box 724 Mayo Memorial Building
University of Minnesota
Minneapolis, Minnesota 55455

Fritz Bach
1965

1990

THE T LYMPHOCYTE PERSPECTIVE OF HLA: A PERSONAL REFLECTION [1,2]

FRITZ H. BACH

Not one of the contributors to this volume could have known, when beginning his or her studies 25 or more years ago, of the magnificent exploration in which each would have the privilege to participate. For those that joined the effort near the beginning, there was surely the hope that understanding of the genetic systems being studied would provide information of both theoretical and clinical import. It was impossible to predict that much more awaited us. In large measure this group would define the major histocompatibility complex in humans, study the remarkable polymorphism of HLA, speculate on and probe the reasons for isotypic complexity in this system, show associations between certain HLA antigens and disease that are as fascinating today as when they were first noted, and enjoy describing the genetic region which was to include factors that may be involved in cell lysis, several of the complement components, and no doubt many yet uncharted genes.

Even Ruggero Ceppellini, that incredible conjectural magician with extraordinary foresight, who contributed so much to this field and who is so very sadly not with us any longer to contribute to this volume, initially involved himself in what was to become HLA to understand more, at the genetic level, of yet another immunogenetic system, as well as, to make a practical contribution to medicine. Even he had no appreciation of the intellectual and practical goldmine that each of us has helped to uncover*.

For me, it was to attempt to "place the homograft reaction into the test tube" that lay at the source of my curiosity. Having attempted to follow the work of Gowans and Medawar during medical school, I was immensely excited during my residency to have the opportunity to hear Peter Medawar give a lecture in New York: he spoke about the then-designated homograft reaction. He posed the issues that we would now refer to as antigen-specificity and the cellular basis of primary and secondary graft rejection. After the lecture, being one of many who had questions for Medawar, I asked him whether he thought that one could ever

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[1] From the Immunobiology Research Center and Departments of Laboratory Medicine/Pathology and Surgery, University of Minnesota School of Medicine, Minneapolis, MN 55455. This work was supported by many grants from the NIH throughout the years of which AI 17687 and AI 22682 are current. FHB is the Harry Kay Chair in Immunobiology. This is paper #546 from the Immunobiology Research Center.

[2] This contribution represents a response to a very special invitation from Paul Terasaki. I have taken the liberty afforded me to write what is clearly not a classical review. I apologize to the many authors whom I do not mention or quote herein and hope that past publications from this laboratory make up for the present omissions.

* Ceppellini, among my very best friends dating back to my spending seven weeks with him in Torino and at his seaside villa in 1963, stayed with us in Minneapolis shortly before his death. I spoke to him at that time about his life and works, telling him that I had been invited to write an article about him for Immunology Today (1). These comments are made based on that conversation. Even at that time, Ceppellini, in his unique, wonderful way, asked me to finish the writing "as soon as possible" so that he could correct it.

elucidate the mechanisms of the reaction that seemed so complex in vivo, as he had described it; did one not have to try to study such a phenomenon in vitro? Medawar, with a smile and a twinkle in his eye that I remember vividly today, urged me to "do just that". Only as I was walking away did I realize what an absolutely presumptuous suggestion I had just made to him. I left the auditorium embarrassed.

In fact, my first effort in "immunology" did involve trying to place the allograft reaction in vitro. In 1963, I was given permission by Lewis Thomas, my chief, to work during my residency in Internal Medicine in the laboratory of Kurt Hirschhorn at NYU. A number of evolving stories were converging at that time. First, there was evidence consistent with the idea that leukocytes expressed histocompatibility antigens. Second, in contrast to the "fact" that I had learned in medical school that lymphocytes were end-stage cells, evidence was accumulating that implicated these cells in a central role in allograft rejection. Third, Peter Nowell had just demonstrated that lymphocytes could respond to phytohemagglutinin by becoming blasts and dividing. This finding was followed by the observations of several authors that a similar response involving blast formation and proliferation would occur if lymphocytes of an individual sensitized to a given soluble antigen (such as PPD or tetanus) were stimulated in culture with that antigen.

I reasoned that mixing leukocytes of two individuals — establishing a mixed leukocyte culture (MLC) — would cause each lymphocyte to respond by blast formation and proliferation to the antigens expressed on the surface of the lymphocytes of the other individual. I appreciated that in vitro responses to soluble antigens such as tetanus or PPD required prior sensitization in vivo. However, this knowledge did not inhibit me from trying an MLC response where there would be no overt prior in vivo sensitization. In fact, in the very first assay in which I mixed leukocytes of two individuals using cell numbers and culture conditions similar to those used in the laboratory to culture cells with PHA or soluble antigens, blast formation and mitosis (Fig. 1) resulted whereas the leukocytes of either individual cultured alone showed no such "response". With that background, Kurt Hirschhorn and I attempted to evaluate whether the degree of response (i.e. the percentage of blasts and mitoses that were seen in the culture) would predict skin graft survival. In what must be regarded as a rather scanty study, we found sufficient evidence to suggest that the MLC might be useful as a test of histocompatibility (2).

I should note that at the same time that I was involved in the development of the MLC at New York University, Barbara Bain and her colleagues noted a very similar reaction (3). Unfortunately, because of the death of Barbara's professor, Louis Lowenstein, the effort in Montreal did not last very long and the MLC and its development and study in large measure was left to a very small group including Pavol and Dagmar Ivanyi, briefly working with Dausset (4), Miggiano and Mattuiz with Ceppellini (5), and Eisjvoogel (whose work I shall come to below); it was, for the next several years, my major effort.

Following one of the first times that I presented the results of the MLC at a meeting in New York, in addition to Gus Nossal's and others' enthusiastic comments, I received a major, but temporary, shock. A man who had been in the audience walked up to the microphone and, after congratulating me briefly on the work, commented that he was glad that I had "reproduced" the findings that he had very recently published relating to the response that takes place when lymphocytes of two individuals are mixed. His name was Robert Schreck. With my German-speaking background this was a rather appropriate coincidence ("schreck" in German means "shock"). In

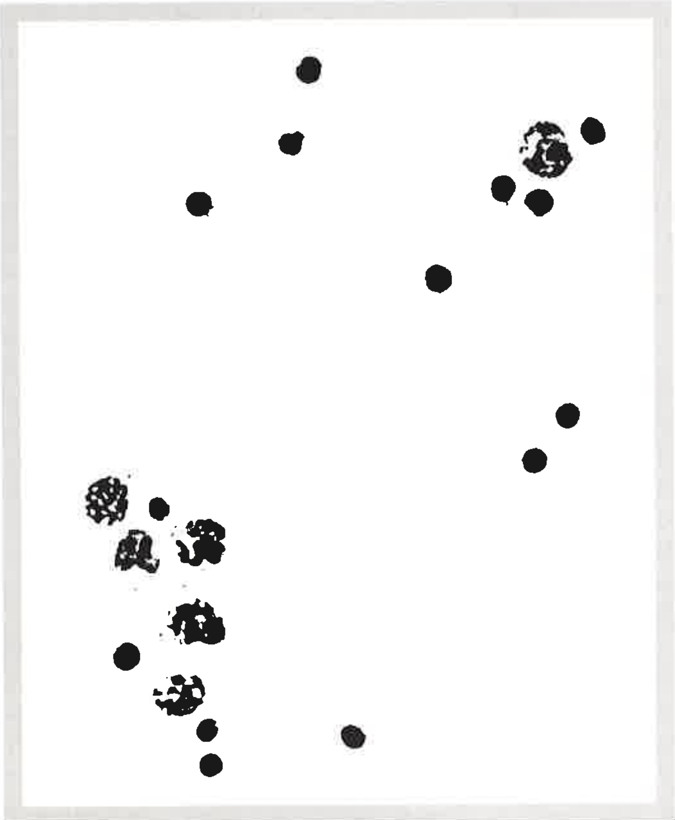

Figure 1. Stained preparation of cells from a mixed lymphocyte culture harvested at 7 days, 14 small cells, 5 enlarged cells, and one mitosis are seen.

fact, what had happened was that his technician had mixed cells of two individuals by error in a study of lymphocyte response to soluble antigens. Since they noted a blast response in the absence of any added soluble antigen, fortunately for me, they decided to discard that particular "mixed" culture from further consideration in their analysis. Barbara Bain, who became a good friend over those years, deserves major credit for recognizing the importance of a similarly serendipitous finding.

In 1964 I still had clinical responsibilities that prevented me from attending the First Histocompatibility Workshop, organized by Bernard Amos and discussed in this volume. Kurt Hirschhorn attended the conference and presented the MLC. I was more than disappointed at not being able to go; I was captivated by the MLC and wanted no part of it to play out without my full participation. I saw the conference as an opportunity to meet investigators whose names, at the time, were only words on publications.

Riding in a taxi in New York with Leslie Brent on the way to a meeting at which I was to present the MLC method, Brent urged me to develop a one-way method of stimulation in the MLC; that way one could probe response separately from the study of antigens that elicit response. I do not remember whether at that time I thought of this potential refinement, but Brent's was a vitally important concept. In fact, I spent the first year (1965-1966) after joining the faculty of the Laboratory of Genetics at the University of Wisconsin, developing such a one-way method. We, as others, tried a number of agents to inactivate what we called the "stimulating cells" so that they could still present their antigens to the responding cells but not themselves contribute to the response. We had by that time switched to using incorporation of radioactive thymidine to detect dividing blasts in the MLC as an assay. It was during an evening of music, for which several of us got together at the home of Charlie Heidelberger (the son of Michael Heidelberger), that Charlie suggested using mitomycin C to prevent the stimulating cells from incorporating thymidine. That method obviously worked well (6). We subsequently found that x-irradiating the stimulating cells gave quite equivalent results to those obtained using mitomycin C-treated cells (7).

I cannot resist telling the story of my very first grant that I submitted around this time and that still continues to support work relating to the MLC. The grant received the highest priority and the budget was left as requested. However, in the critique the reviewers commented: "We hope that the applicant, who appears to be a promising young investigator, does not become too enamored by this new test of his, the mixed leukocyte culture, which clearly has very limited applicability".

During my first year at Wisconsin, I met a woman who worked as a part-time secretary who, incidentally, could type far more than 100 words per minute. She needed that level of efficiency for she had 17 living children plus husband. We became friends and she volunteered her family as blood donors for our studies. That family provided all the material one could have hoped for: each of the four possible patterns of segregation of the HLA chromosomes was found with at least two siblings representing each genotype.

One item that considerably disturbed me was that I did not have much research money available to pay the family as donors. In fact, my only funds were $8,500 from the Damon Runyon Foundation for the year and a small amount from the University of Wisconsin. My memory is that I paid them $5 per visit for 19 bloods. Fortunately, at a social for new faculty members, I met a newly-appointed professor of psychiatry. He was enormously interested in my development of a test of compatibility and asked for many experimental and conceptual details. It was clear to me that the University of Wisconsin had hired a new faculty member of great vision and depth. The jolt came fifteen minutes later when he asked to interview the family and subsequently evaluate whether the mixed leukocyte culture predicted emotional compatibility. I felt sure the Hildebrandts would willingly cooperate but told the psychiatrist that I felt they would have to be adequately reimbursed. Clearly he was much more grant (or personally) rich than I: he paid them each $50 per interview and interviewed every member of the family six times: it salved my conscience. I had discouraged my colleague from the project but was torn by my hope that the family would finally receive some significant remuneration for their wonderful generosity and warmth (I had breakfast at their house every time I went there at 5:30 a.m. to draw their bloods). The results of his study were predictable and I used the lack of correlation that he uncovered when I taught statistics to medical students.

Ceppellini and others were at that time analyzing the results of skin graft survival between individuals of various genetic relationships (5). Their approach was to choose a "cut-off" based on times of skin graft rejection in a relatively small panel of unrelated individuals. They then considered graft survival in other genetic combinations as "compatible" if grafts survived longer than predicted by the studies of the unrelated individual pairs. The genetic "solution" (the number of loci and alleles that controlled skin graft rejection), of course, depended on the cut-off. Times of skin graft rejection in compatible versus incompatible pairs, however, did not fit a biphasic distribution but rather represented a continuum of incompatibility. We were more fortunate: stimulation and non-stimulation in MLC were statistically distinguishable responses.

We noted in applying the one-way method that about 25% of sibling pairs did not stimulate in MLC while MLCs between individuals of any other genetic relationship virtually always showed a response. Our first findings, in fact, were that approximately 25% of MLCs between brothers did not stimulate whereas all MLCs between sisters did; the data supported a p <0.01 for the male association of non-stimulation. Fortunately, while writing a paper to which many of the faculty in the Laboratory of Genetics at Wisconsin had contributed ideas to explain this male association of identity in MLC, which was great intellectual fun, we began to find non-stimulatory sister pairs.

These findings made me think that a genetic analysis might be done for reactivity in the MLC based on the finding of non-reactivity in a given percentage of sibling pairs but not in other genetic combinations. In discussions with Jim Crow, Chairman of the Laboratory of Genetics, and the brilliant mathematical geneticist Charlie Cotterman, I learned a great deal about the difficulties of doing genetic analyses in humans. One remarkable session with Charlie lasted through the night (for which he was noted); he spoke in a manner that preempted the possibility of ending the conversation. That session was a windfall, for I learned so much from him in that 6 p.m. to 7 a.m. "conversation". In fact, when we did the analysis in early 1966, i.e. probing the genetic control of reactivity in MLC, we noted that a single genetic "locus" (in a transmissional sense) with 15 or more alleles (based on several assumptions including equal allelic frequencies) appeared to control reactivity in MLC (8). This was not a bad estimate. In fact, recognizing that stimulation in MLC is controlled by class II antigens, and given the very great immunodominance of the DR antigens, we do deal with approximately twice that number of (DR) haplotypes, of quite unequal frequencies, in the population.

Preceding and concurrent with my efforts at establishing a cellular method of histocompatibility testing were the efforts of many laboratories in developing the serology of HLA: the sole or major basis of the work at that time of my several colleagues who have authored chapters for this book. These serologic studies had by this time already pointed to the fact that the antigens in question were histocompatibility antigens (9). Bernard Amos and I, in many immensely enjoyable discussions, thought that perhaps we should compare reactivity in MLC (again no response versus a positive response), with identity or difference for the serologically-defined antigens. In a collaboration of several months, during which time Bernard and I, as well as cells and sera, travelled to each others' laboratories, we noted that there was an exceedingly high correlation between the results of non-reactivity in MLC (in the 25% of sibling pairs in which it was noted) and identity for the great majority of serologic determinants recognized with the antisera then in use (10). In mouse it was the H-2-encoded antigens that appeared to account for reactivity in MLC and we used this knowledge to bolster our reasoning that the locus controlling reactivity of MLC in humans was the homologous genetic system (it was only later that Festenstein and colleagues described the strongly-stimulating determinants of the Mls locus). There was also work documenting that determinants that elicit antibody responses in other species are those that are encoded by the MHC. Our paper was accepted rapidly by the editors of Science with only a single request: they asked that we give them a p value for the correlation that we had found (identity in 177/185 comparison made). The p was so ridiculously low that Bernard and I persuaded the editors to settle for the statement: "The probability of obtaining the correlation we have found (177/185) by chance alone is vanishingly small".

Bernard and I discussed our finding that the same genetic system appeared to control both reactivity in MLC and the serologically-detected determinants in the context of two related observations. First, that both reactivity in MLC and determinants that readily engender antibody response are encoded by the MHC in experimental species. Second, that the MLC, as well as serologic typing, appeared to predict graft survival. We reasoned that we were, in fact, defining the major histocompatibility complex in man (10). At that time, in 1967, we used the term Hu-1 because Dausset had already used that designation for the locus controlling the serologically-defined antigens he was detecting; we did not want to add to the terminologic confusion in the field.

van Rood and Dausset had more than just an inkling that they were dealing with a single genetic locus and, more important, they suggested that this may be the major histocompatibility locus in man. Bernard and I felt that combining serologic testing with the MLC, which I thought might represent the in vitro model that I had brazenly proposed to Peter Medawar, provided a sounder biologic basis for the definition of the major histocompatibility complex in man.

In what turned out to be one of the most exciting dissections of the genetic control of MLC reactivity related to HLA, Bernard and I found one exception in our study. Cells of a sibling pair that appeared identical by serologic testing did stimulate in MLC. Over a bottle of very good Scotch whisky, sitting on the desk in my study at home in Madison, Bernard and I discussed possible explanations. One possibility that we advanced to explain the finding was that there was a part of the MHC in humans different from, but linked to, the genes encoding the serologically-defined antigens that might be of importance in stimulating the MLC response (11). While we had no direct evidence in that regard, that hypothetical region turned out to be HLA-D.

If the MLC were to be used as a histocompatibility test for transplantation, those in my laboratory in 1967 realized the necessity for quantifying the test in a biologically meaningful manner. In Madison, Richard Albertini, my first post-doctoral fellow, and I developed conditions that allowed quantitation by showing, in family studies, that responses in parent-child combinations and in siblings that differed by one haplotype were lower than responses between siblings that differed by two haplotypes (12).

When I presented this work at the Torino workshop later that same year, Ceppellini was outspokenly doubtful that such quantitation could be accomplished. In one way this was not unlike the incident when I first met Ruggero at a symposium in which I gave a talk at the International Genetics Congress in the Hague. After that talk he complimented me but expressed doubt about my findings; our interchange was followed by an invitation for me to visit him in Torino; that was in 1963. Ceppellini also invited me after my talk at the Torino workshop in 1967 to what would be the only triple-blind study in which I shall ever be involved. Dick Albertini and I travelled to Torino to study the families of The Third International Histocompatibility Workshop. Ceppellini decided that he did not even want to be in Torino when we arrived nor during the course of experiments because he held the ultimate code which would tell us whether we could really predict reactivity quantitatively. Miggiano and Curtoni were each given certain information, but even they knew no more than to give us the coded samples. Only after the experiments were finished and the results written down and given to Ruggero's secretary, Anna, did Ruggero return to the laboratory to pass final judgment. Quantitation was a success with only one exception: again, cells of one sibling pair that typed identically from a serologic perspective, did stimulate in MLC. Ceppellini liked the idea that Bernard and I had advanced suggesting the possibility that an additional locus of HLA may be of great importance in stimulating MLC responses, i.e. HLA-D (13). Ed Yunis and Bernard Amos, studying a family from Minneapolis in which one individual inherited a B-DR recombinant haplotype, subsequently provided convincing data for the existence of the separate region that was of greatest importance for stimulation in MLC (14).

It was in 1968 that one of the most rewarding experiences of my career took place. I had met Dr. Robert Good when he interviewed me in 1963 for an Advanced Research Fellowship of the American Heart Association. He arrived at his office 20 minutes late during which time I had the expected palpitations. He entered with that boundless energy that characterizes him, smiled broadly, took me into his office and spent less than a minute to assure me that he thought my proposal was excellent, that I would get the fellowship, and that he wanted me to relax so that he could learn more immunogenetics (the topic of my proposal). We spent the ensuing 18 hours together, literally without parting, ending the day in his apartment at 4 a.m. Good guided me along the trail that he blazed for the whole medical community. He told me how he thought about children with immune deficiencies as "Experiments of Nature" and how he felt that understanding these childrens' deficiencies would help us to understand immunology. The remarkable vision of this truly brilliant clinical investigator led to a series of collaborative investigations that resulted in many believing that I had worked with Bob; in fact, I never did.

However, we did interact intensively on several occasions including a workshop on immune deficiency disease, the proceedings of which were published in 1968, at which the concept of bone marrow transplantation in humans was discussed. Hilaire Meuwissen, then working with Bob, came to my laboratory in Madison to learn the MLC method. In mid-1968, while an assistant professor at Madison, I received a call from a pediatrician at Columbia Presbyterian Hospital in New York asking me whether I would consider doing a bone marrow transplant on a 23 month old boy with Wiskott-Aldrich syndrome. It turned out that the referring physician had first called Bob Good, but that Good had declined to accept the boy as a patient. However, Good did tell him that he should call me and perhaps I would consider the procedure. After some hasty reading, I agreed to attempt to match the patient, David Zeissett, with his sister. They were identical in MLC. Together with Mortimer Bortin, until then a bone marrow transplanter in mice, we performed what became one of the first successful, matched human bone marrow transplants (15).

The other successful transplant was done in Minneapolis by Good and colleagues. That patient, with severe combined immunodeficiency disease, also had a potential sibling donor. However, the patient was mismatched with the donor for one class I antigen. Bob called me to discuss this situation because of his concern that one may not want to do the transplant given the incompatibility. I suggested to him that identity in MLC would be important to assess; subsequently, we tested the bloods of the patient and the potential donor in Madison (I believe they were also tested in Minneapolis by Hilaire Meuwissen), and found MLC non-reactivity. That patient was also transplanted. Independently, Jon van Rood was involved with van Bekkum and DeVries in doing a matched transplant, also in a patient with severe combined immunodeficiency disease.

The exciting work of Teddy Brunner prompted my interest in cytotoxic T cells. It seemed not unreasonable to think that such cells might be generated as a part of the proliferative response that we were measuring in MLC. A technician in the laboratory who is now Professor of Immunology in Houston, Susan Solliday (Rich), and I demonstrated that this did, indeed, occur. In 1970, the same year that Svedmyr and Hodes (16) as well as Hayry and Defendi (17) published their assays in mouse, we published ours in humans (18), showing antigen-specificity of the cytotoxic cells generated in MLC. Some years later, Ceppellini, with Vincenzo Miggiano and Jim Lightbody, referred to this assay as the cell mediated lympholysis (CML) assay. For me, however, the greatest importance of having an assay for the development and testing of cytotoxic cells was yet to come.

One of the more comical events that stands out in my mind related to the Histocompatibility Workshops occurred at the Histocompatibility Workshop in Los Angeles in 1970. Marilyn Bach and I attended; one night after going to sleep around 2 a.m., after a party that I am sure is still remembered by many, I awakened only to find a man in the room. He was perfectly attired with dark blue suit, white shirt and conservative tie. I, naked (a significant detail in the story), jumped out of bed to find him bending over Marilyn. Marilyn, nonetheless was laughing; I, on the other hand, having lived in New York for several years, not only saw no humor but was ready to do battle. As we started to speak it turned out that our visitor was German (we switched to that language) and was lost. No struggle ensued; rather, I called to get a room number for him and, with that information ushered him out of the room. After a heated discussion with Marilyn, in which she pointed out that given his dress and mannerisms it was obvious to her that he was quite harmless, we went back to sleep.

The next morning we joined a group at breakfast, including Jon. Great levity pervaded the conversation. When I asked what was so funny, I was told that this poor young German investigator present at the Congress had become completely inebriated the previous evening, had locked himself out of the hotel on the eighth floor by going out a fire escape door that locked behind him, and then had to crawl along a 1-foot wide ledge until he found a room with an open window. He apparently had told his friends that the only thing that he remembered beyond that was that the man in that room had jumped out of bed and very aggressively confronted him. Further, that he did not know who the man was except that he was "all covered with hair". At this point, one of the individuals at the breakfast table laughed uproariously and said: "But, Michael Feldman isn't at this meeting". The tale was told often after that with Marilyn and myself having perhaps the funniest memories of it.

With the turn of the decade, having worked up to 1970 in humans, I felt I should attempt to take advantage of the recombinant inbred mouse strains that were generating so much interest. After initially approaching Don Shreffler, who unfortunately could not commit to a joint project at that time, I approached Jan Klein to suggest a collaboration to probe which regions of H-2 were of greatest import in controlling stimulation of proliferation in MLC. Klein provided us with the initial strains that we tested; later Stimpfling, Shreffler, Bailey and others were generous contributors of special strains.

In today's world of the MHC, in which so much attention is given to the class II genes, their antigens and their function, it must be difficult to realize the excitement and joy of discovery that Michael Widmer (initially a technician in the laboratory but now a well-known, highly-productive scientist) and I, with Miriam Segall and Marilyn Bach, felt in 1971 and 1972 as we analyzed the results of our studies, only to find that it appeared to be disparities for the H-2 I region that resulted in the strongest responses in MLC (19,20). McDevitt and colleagues mapped immune response control to the I region. No one knew, however, that there were potential histocompatibility antigens encoded by genes in this region, and that the region and its products played a role in transplantation immunity. We suggested that the antigens encoded by the H-2 I region that were detected in MLC may be the same as the Ir gene products, but this was mere speculation.

Studies of additional strains, which were paralleled by experiments of Meo and Shreffler (21), showed the general dictum to hold true: the class II antigens appeared most important in stimulating proliferation in MLC. It was around the time of these studies that we began to use the term "major histocompatibility complex" (MHC) to reflect the fact that transplantation immunologists were dealing with a complex series of loci in relation to studies of histocompatibility.

In 1971, as the above story was unfolding, I became intrigued with antigens that were difficult to define serologically but that seemed to provide such an important stimulus to alloreactivity of T cells. (At that time there were no anti-Ia sera in mouse or humans). Thus, in addition to using the abbreviation "MHC" as the term for the whole genetic region, we introduced the label SD to refer to the then serologically-defined antigens and epitopes (now referred to as class I antigens) and the label LD (lymphocyte-defined) for those antigens recognized by proliferating T cells (now referred to as class II). This terminology was used to emphasize that there are two classes of antigens encoded by MHC genes that subserved different functions. In addition, as more data were obtained, the LD-SD concept included the finding that determinants recognized on the class I and class II products by antisera were different from those recognized by T cells.

Following some comments I made while chairing a workshop at the First International Immunology Conference in Washington, D. C. in 1971, Donald Bailey approached me to ask whether I might have interest in studying his H-2 mutant, then referred to as H(z1) and now as bm1. Despite extensive efforts, no antisera had been raised to detect the difference that led to the rather rapid reciprocal skin graft rejection seen between mice of that mutant strain and its parent, C57BL/6. We tested proliferation and generation of cytotoxicity in the C57BL/6 anti-H(z1) combination and found not only proliferation but also very strong generation of cytotoxicity, thereby providing in vitro data showing that the genetic alterations involved in the "H-2 mutants" could be highly immunogenic to T cells (22). These findings were very influential in my thinking regarding the differences in epitopes seen by T cells versus those recognized serologically. It was the study of those polymorphisms that are easily detected by T cells and not so readily by antisera, as is found with these mutants as well as the T cell-defined Dw subtypes of HLA (which I shall discuss below), that I emphasized in my approach to obtain information about the structural basis of T cell recognition.

When Dolores Schendel came into the laboratory as a student for the Ph.D., she chose to work with Barbara Alter, my close co-worker and dear friend of more than twenty years, on studies of the genetic control of cytotoxicity. From that work arose what, to me, were perhaps among the most exciting and important contributions that came from our own studies as they relate to HLA. Dolores and Barbara confirmed that the great majority of the proliferative response was to allogeneic differences encoded by the H-2 I region, i.e. class II antigens, but showed that the strongest cytotoxic response was against H-2K and H-2D, i.e. the class I antigens (23-26).

A ten-day period in 1972, immediately prior to the International Transplantation Conference to be held in San Francisco, was literally packed with new data, speculations, and designing of models, including the beginning of the model that later laid the basic rules for response of cytotoxic T cells (27). I hosted a very small workshop in Madison on cellular response to HLA during that time. I presented the data dealing with the mutants at that conference and Dolores and Barbara planned to culture cells of the recombinant inbred strains to test the relative roles of response to class I and class II antigens in the generation of cytotoxicity using recombinant inbred strains. I did not, however, expect anything like the results that Vincent Eisjvoogel (who had come to

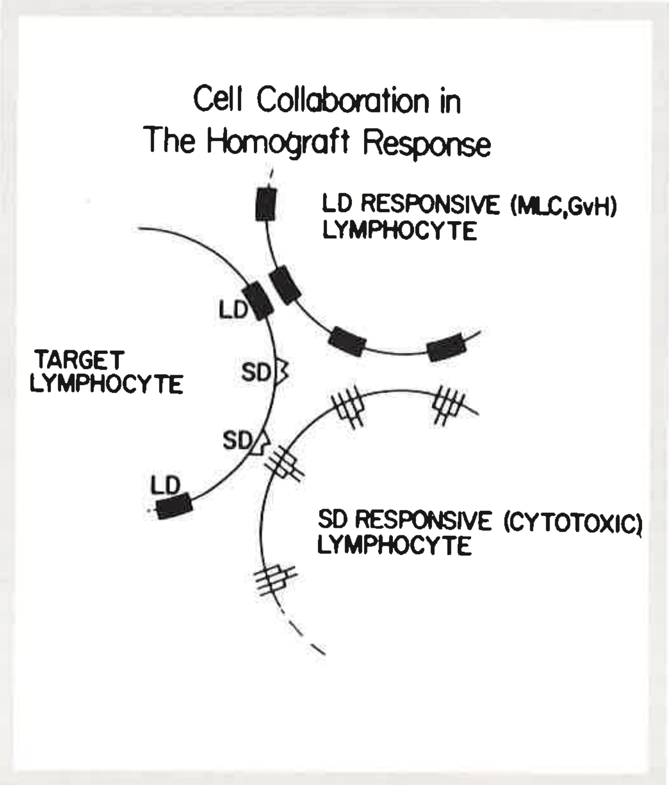

Figure 2. A schematic representation of a cellular model explained in the text.

Hirschhorn's laboratory to learn the MLC during my stay there) presented: he showed us, in studies of one human family, that generation of cytotoxicity against class I antigens was dependent on the presence of a class II disparity on the stimulating cells (28). What did not exist was an explanation.

We continued, over the next days before leaving for San Francisco, to wrestle with our own accumulating findings and the data presented by Eisjvoogel. It occurred to me that the most likely model was that the cells responding proliferatively to the class II antigens were different from the cytotoxic cells responding to the class I antigens. Given the findings in humans and those that we subsequently obtained in mice, i.e. that the generation of the cytotoxic cells appeared to require the class II-directed proliferative response, I suggested that two separate, functionally-disparate populations of cells were likely involved: class II-reactive cells that were helper T lymphocytes (Th) and that provided help to the class I-responsive cytotoxic T cells (Tc) (29).

Days later, in a plenary session address at the San Francisco meeting, with newly-made slides, I proposed that there was a dichotomy of function for what are now called class I and class II antigens in that the class II antigens stimulate Th and that the class I antigens stimulate Tc. Further, there was collaboration between the two cell types, i.e. Th-Tc collaboration (29). I summarized these ideas in a figure in that article (Fig. 2). The picture of cell collaboration in the homograft response suggested that functionally disparate subpopulation of lymphocytes respond to class I (referred to in the figure as SD) and class II (referred to as LD) antigens. The listing of MLC together with GvH in parentheses after "LD Responsive", referred to the proliferative response in the MLC being one measure of response of Th to LD alloantigens. Indeed, proliferation in lymphocyte cultures, including the MLC, is still widely interpreted as a response of Th, although many caveats must be recognized given our present knowledge when making this extrapolation. GvH referred to splenomegaly, the Simonsen assay, or to lymph node enlargement.

In that same paper I included the cartoon of the "Sea of Confusion" (Fig. 3). I intended with this latter figure to point out that

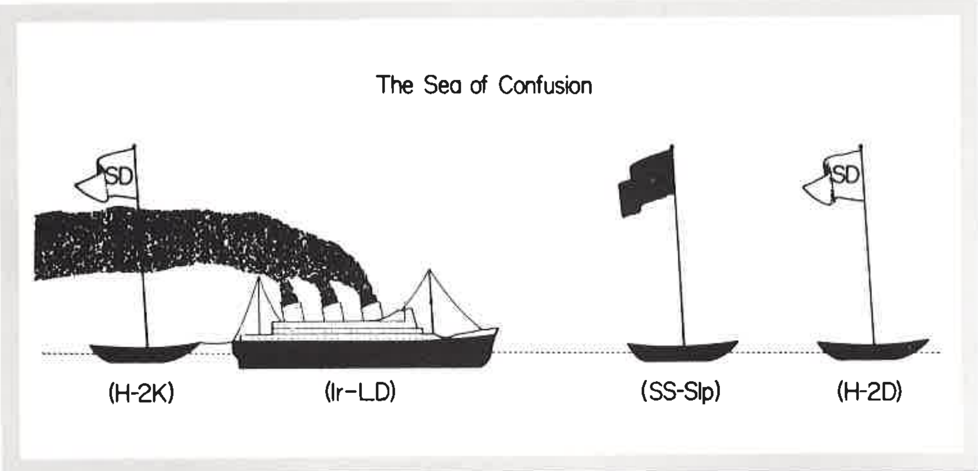

Figure 3. A cartoon of the MHC-ms. Details explained in the text.

much of what we saw as reactivity to the MHC was related to a region that had not yet come to our attention in terms either of encoding antigens or potentially playing a role in graft rejection.

Much of the next three years was given to elucidating the relationship between what are now called the class I and class II antigens and the response of Th and Tc. These studies included the use of the three-cell protocol in which the class I antigens were present on one set of stimulating cells and the class II antigens on a second set of stimulating cells (23-26). Also, the presumed Th and Tc were separated on monolayers (30), so that the model proposed could be tested. It is worth noting that once again transplantation immunology provided information for what was to become cellular immunology: all of these data were published before Zinkernagle and Doherty showed the difference in response of Th and Tc to virus presented by class II and class I antigens.

It was during this period, in 1973, that I organized the only conference that I have undertaken myself. It was in the south of France just a few kilometers from the Cote d'Azur in St. Paul de Vence at a newly-built hotel, the Mas d'Artigny. We enjoyed the equivalent of multi-star cuisine and individual swimming pools for many of the rooms. (I felt obliged to spend one week prior to the conference with my family at the hotel to make sure that all was adequately prepared.) For cellular immunogeneticists of the MHC (or "cellology" as Jon van Rood later called it), this was an extraordinary time. Concepts relating to what would be called the class I and class II antigens, but at that time still referred to as SD and LD, were coming together; the potential relationship of the class II antigens and Ir genes was manifest, and the relationship of all this to transplantation was discernible. The proceedings were published in Transplantation Proceedings and later in a bound volume (31).

In the early 1970's, there was discussion within the transplantation community of the need for a faster MLC test that would provide a rapid cellular "crossmatch" and help dissect the increasingly apparent complexity of the HLA-D region. At a meeting in Milano, in 1974, Jean-Charles Cerrottini presented his data on the generation of a secondary cytotoxic response in vitro. He showed data in mice demonstrating that if responding cells in an MLC are primed in vitro to a given allogeneic stimulus and allowed to "revert" to cells that no longer had cytotoxic potential over a period of approximately two weeks, then addition of the same allogeneic stimulus led to the appearance of a secondary response that was much faster than the primary. I asked Jean-Charles after his lecture whether he had ever looked at the proliferative response with the same approach: he had not. Marilyn Bach, who was my wife and colleague at that time, was with Jon van Rood in Leiden doing some experiments, but was due to return to Madison the next day. I called her, told her of the experiments of Cerrottini and asked her to arrange to have someone in Madison set up similar culture in humans to see whether a more rapid secondary proliferative response resulted. I felt confident that such a response, if it occurred, would be against the class II antigens and might thus help with a more rapid cellular test for the class II antigens.

Experiments moved rapidly. Michael Sheehy and Paul Sondel, at that time both Ph.D. students, collaborated to describe the primed lymphocyte (LD) typing (PLT) test (32) for which Rudy Wank did the serologic typing and Karen Zier, also a Ph.D. student, contributed to the study (33). In the PLT, the lymphocytes responding in the primary MLC were left in culture for several days after their peak proliferative response so that they would "revert", i.e. become non-dividing small lymphocytes again. These primed cells, mostly representing responders to the alloantigens on the specific stimulating cells, then showed a much more rapid response when confronted again with those same antigens. Fradelizi, Mawas, Sasportes and colleagues also demonstrated a secondary response in human cultures (34). These studies not only provided a method that still has utility especially in the definition of the DP polymorphism, but also led to the cloning of human T lymphocytes (35).

It was in the middle 1970's that I was asked by the Editor of the New England Journal of Medicine, Franz Ingelfinger, to write a three-part review of HLA. The invitation was flattering. While accepting with no small degree of pleasure, I asked whether I could invite Jon van Rood to co-author the article with me to represent the serologic perspective. Jon and I spent many hours over a period of more than a year in our respective houses, offices, and on his boat. We had a wonderful time assembling the review. We drank champagne on the day that we sent it off. It took only two weeks for Ingelfinger to return the review with a terse first sentence: "To get right to the point, I must tell you that the review you have written is not suitable for publication in the New England Journal of Medicine". A five page letter followed. It took but two minutes for me to call to discuss this issue with him; I ignored my usual dictum to sleep on anything like this before responding. Ingelfinger told me that it was the style and not the overall content (although he asserted that we had included too much in the way of specialized details and terminology) that was troubling the editors, and would Jon and I be willing to re-write the paper.

After much conversation across the Atlantic, Jon and I set to work again. We sent in the revised manuscript, that we felt was truly much better for the readers of the NEJM, and drank another bottle of champagne, this time in Leiden. It took only days: Franz Ingelfinger called me to tell me that the paper was still not suitable but, before I could say one word to him, he suggested that I come to Boston and that he would sit with me to rewrite the manuscript. In a remarkable day that reflected the exceptional man that he was, he, then dying of cancer, discussed not only literary style but also often asked me to elaborate on certain points in greater detail than Jon and I had covered in our article. We did rewrite the paper and it was published (36), although Jon and I waited until we read it in the NEJM before drinking that third bottle of champagne. Jon and I look back on the many discussions that we had with great pleasure and on the overall episode as one that, in retrospect, embodied great humor and delight. Writing a review is, for me, a wonderful experience, full of long discussions and intellectual flights of fancy that are allowed at these times; I think primarily of one other article: the one with Paul Sondel and Marilyn Bach (27).

It seems almost improper for me to continue the story beyond this point. By the mid-1970's, it was no longer the saga of a few individuals who had the great good fortune to nurture this science in its infancy, but rather a tale of many. In my own area, approaching HLA from the T cell perspective, there were the several individuals who worked on the development and testing of homozygous typing cells and others who were productive in diverse areas. To mention just a few: Wolfgang Mempel, who spent time with me just prior to returning to West Germany and having the idea to use HTCs, Bo Dupont, Eric Thorsby, Ben Bradley (with van Rood), Marilynne Sasportes, Arne Svejgaard, Fritz Jorgensen and Claude Mawas. Nonetheless, allow me to continue one thread of my adventure.

I was fascinated by the LD-SD dichotomy, particularly with the presumption that the determinants seen by T cells were largely different from those seen serologically. To the extent that this construct had validity, it seemed to me that there was the need to define the LD polymorphism to match for transplantation, since it appeared that rejection was highly associated with T cell response to HLA antigens in vivo. Our study of the H-2 mutants, among many other lines of evidence, had convinced me of this role for the LD polymorphism in T cell immunity. Thus, as it became clear that there was a polymorphism associated with the class II antigens that could be seen over and above that recognized serologically, i.e. the definition of the Dw subtypes, I urged several students and post-doctoral fellows to become involved in these studies. The studies, done in the five year period from 1980 to 1985, were among the first that we performed after the move to the University of Minnesota.

Nancy Reinsmoen performed what was the most extensive definition of the Dw subtypes of DR4 (including Dw4, Dw10, Dw13, Dw14 and Dw15) using HTCs (37) and later performed similar analyses with respect to DR2 (Dw2, Dw12, Dw21, which we referred to as MN2, and Dw22) (38). While these studies provided further evidence that the LD polymorphism is more extensive than, and subtypic to, the serologically (SD)-defined polymorphism, the studies with HTCs did not prove that the LD polymorphism was related particularly to any one of the class II products.

Thus, Jeffrey Groner, a surgical fellow in the laboratory, and Andy Watson provided proof at the protein level that DR β chains showed a protein polymorphism detected by isoelectric focusing that correlated with the Dw subtypes. Fortunately, we chose to study the DR4 haplotypes so that the DR $\beta 1$ product was sufficiently prominent to allow us to find that polymorphism (39). Toshio Yabe and Nancy Reinsmoen extended these studies to DR2 (40), at the same time that Beth Morley-Lee, who began her studies in my laboratory but then moved to Stanford with her husband to work with McDevitt, obtained similar results.

Availability of the restriction fragment length polymorphism (RFLP) testing procedure raised an interesting question: was the degree of RFLP seen with haplotypes such as those encoding the various Dw subtypes of DR4 as great as what several laboratories had described for the haplotypes encoding different DR antigens? Janice Nicklas, Harriet Noreen and Miriam Segall found that there was essentially no detectable RFLP associated with the different DR4 haplotypes (41). This observation led us to speculate, rather academically, that the DR4-associated Dw subtype polymorphism may be special among the total HLA class II polymorphism in that it may have arisen very recently in evolution, perhaps after a population bottleneck (42).

Obviously, the next step was to sequence the genes encoding these proteins. Scott Cairns and Carol Dahl, then post-doctoral fellows, provided the first DNA sequence information relating to the Dw subtypes and showed, in that study, the obvious importance of amino acids 67, 70, 71 and 74 (among others) of the DR β chain in T cells allo- and restricted-recognition (43). And, finally, Shoakee Wu, who over a period of years was a co-advisee for the Ph.D., a post-doctoral fellow and finally a colleague appointed as an assistant professor in the Immunobiology Research Center (the name of our academic unit), provided the first DNA sequence information for the DR β1 and DR β3 genes of the Dw subtypes associated with DR2 (44). In the process, Shoakee found an example of what we thought might be reciprocal gene exchange between DR β genes.

I tell this evolving LD/Dw subtype story for several reasons. Of course, there was the pleasure of providing data very early for the phenotypic (HTC), protein, RFLP and finally DNA sequence levels of analysis. More than that, however, these studies retrospectively explained many of the findings discussed above. I was wedded to the idea that we would provide additional information for the field by studying T cell responses to HLA antigens. (I shall not identify the author of the comment (although he is one of the contributors to this volume), who said to me "you just cannot compete with serology using your laborious T cell methods".) I was comfortable with the idea that this was not a competition but rather a quest for complementary information.

Today, many have woven their individual designs and contributed them to the tapestry that reflects our understanding of HLA. And yet even today, I am convinced that the "functional approach" of using T cells will provide great excitement to all those bright and wonderful young investigators who now lead the field. There is the issue of adaptation of the host to the graft in both solid organ transplantation and in bone marrow transplantation. There is the possibility that different class II antigens (DR versus DQ, for instance) may differentially activate functionally disparate subpopulations of cells. There are the enigmas of HLA and disease association, not only for autoimmune diseases but also for conditions such as narcolepsy, which is such a dramatic and challenging finding.

In a more general sense: there are yet many uncharted paths that deserve investigation. Whether those areas will yield for their devotees the rewards that some of us have enjoyed participating in the HLA saga cannot be guaranteed. If I were to venture a guess in that regard, I would have faith that this genetic region has in it much of intellectual and practical importance still to be unraveled.

During these twenty-six years I have had the opportunity to interact professionally and personally with some extraordinary individuals. Of my colleagues, Ruggero Ceppellini and Jon van Rood stand out most. It is these two individuals who, from the beginning, were most supportive of the value of continuing MLC studies. It may be difficult today, given the very wide usage and consideration of the MLC, to understand the lack of notice and in some cases overt criticism of the need for further studies with the MLC, that existed in the early days.

Ruggero was brilliant, charming, witty, and outspoken on every subject. The editor of Immunology Today allowed me to write a personal tribute to him after his death in which I had the opportunity to expand on his phenomenal attributes (1). There are so many stories that should be told of Ruggero. One that I included in my article in Immunology Today concerned an invitation that was issued him to chair and speak at a session at the Histocompatibility Workshop hosted by Kissmeyer. He turned down the invitation. Marilyn Bach then wrote him an obviously persuasive letter, for he answered: "I shall be there — noblesse oblige". That was his style. He took for himself, and deserved, that place in our field.

Jon is possessed with extraordinary insights, sound advice (scientifically and personally) and above all, humanity. He has the immense stature of a great academician in belief and action. He and his family are our closest of friends; he and I have had the most wonderful of friendships. His sense of humor never fails him: after one lecture, late in the 1960's or perhaps in 1970, during which I was trying as hard as I could to pronounce Jon's last name in the Dutch style (which ends up with a pronunciation quite different from the usual pronunciation used outside of Holland), Jon accused me of purposely pronouncing his name in a way different from how people were used to hearing it so that he would not get the credit for whatever I had said about him.

I must add Bernard Amos. He is a special man, who, when we did have interactions (which were all too infrequent after our collaboration in the late 1960's), was remarkable in many ways.

Of my coworkers and students, there is what appears to me an endless list of bright, productive, ebullient individuals. While I would like them all to be listed here, that is impossible. Paul Sondel stands out. He is endowed with intellect, generosity, and altruism. His contributions to the story that I have tried to tell above were many and important. Barbara Alter, with whom I interacted on a daily basis for so many years; Miriam Segall, who is a wonderful friend and an "intellectual resource" for me (she was my student and is now my colleague); Marilyn Bach, who made manifold contributions from so many perspectives; Dolores Schendel; Michael Sheehy; Dick Albertini; Susan Solliday (Rich); Michael Widmer and others deserve much of the credit for the progress we did make and most of the credit for making this such an enjoyable foray for me. And so much credit goes to Genia Gordon, who has taken responsibility for many of the tasks that keep this laboratory functioning. She has done brilliantly, first at the University of Wisconsin in the Laboratory of Genetics and the Immunobiology Research Center, and for the last decade in the IRC in Minnesota.

I have had unequaled support at all levels from those personally close to me. But of these, it is my remarkable children that stand out. They understood my commitment. They kept their sense of humor at times that strained imagination. And, in a matchless way, they were always there to provide succor or solace, and to partake in moments of joy. Even with this manuscript, my younger son, Peter, while sharing the moment of reflection, has provided delightful and salutary editorial comment.

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CURRICULUM VITAE

Fritz H. Bach

PERSONAL

Born: April 5, 1934
Place: Vienna, Austria
Children: David, Peter, Wendy, Kathryn, Erika
Home Address: 34 Park Lane, Minneapolis, MN 55416
Office Address: Immunobiology Research Center
Box 724 Mayo Memorial Bldg.
University of Minnesota
Minneapolis, Minnesota 55455

EDUCATION

	Undergraduate College
1951-55	Harvard University, Cambridge, Mass. A.B.
	Medical College
1956-58	Washington University School of Medicine, St. Louis, Mo.
1958-60	Harvard Medical School M.D.
	Internship and Residency
1960-63	Internship, First and Second year Medical Residency, Third and Fourth (New York University) Medical Division, Bellevue Hospital, New York, New York

TEACHING AND RESEARCH APPOINTMENTS

1955-56	Research Associate, Beth Israel Hospital, Boston, Mass.
1963-64	Genetics Traineeship (USPHS), New York University Medical School, New York, New York
1964-67	Advanced Research Fellowship, American Heart Association
1967-73	Established Investigatorship, American Heart Association
1965-66	Instructor in Medicine, University of Wisconsin, Madison
1966-69	Assistant Professor, Departments of Medical Genetics and Medicine, University of Wisconsin, Madison
1969-72	Associate Professor, Departments of Medical Genetics and Medicine, University of Wisconsin, Madison
1972-73	Associate Professor, Departments of Medical Genetics and Surgery, University of Wisconsin, Madison
1973-80	Professor, Departments of Medical Genetics and Surgery, University of Wisconsin, Madison
1974-79	Director, Immunobiology Research Center, University of Wisconsin, Madison
1979-	Professor, Departments of Laboratory Medicine/ Pathology and Surgery, University of Minnesota, Minneapolis
1979-	Director, Immunobiology Research Center, University of Minnesota, Minneapolis

1984-	Harry Kay Chair in Immunobiology, University of Minnesota, Minneapolis

PROFESSIONAL SOCIETIES AND AWARDS

	American Society for Clinical Investigation
	American Association of Immunologists
	Transplantation Society
	Established Investigator of the American Heart Association
1974	Modern Medicine, Distinguished Achievement Award
	Sumner Memorial Lecturer
1977	Milwaukee Academy of Medicine, Distinguished Achievement Award
1979-84	Leukemia Society of America, Nat'l. Board of Trustees
1983	American Red Cross, Distinguished Scientist Award
1984	Medal of the College de France
1985	Weizmann Institute, International Board of Governors
1987	Foreign Member, Royal Dutch Academy of Sciences
1989	The Emilio Trabucchi Foundation Award
1989	Charter Member, International Bone Marrow Transplant Registry

EDITORIAL BOARDS AND EDITORSHIPS (Past and Present)

Proceedings of the Transplantation Society
Transplantation
Tissue Antigens
Journal of Immunology
Journal of Clinical Immunology and Immunopathology
Annales d'Immunologie
Human Immunology
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Immunology Today
Journal of Molecular Biology and Medicine
Editor, Clinical Immunobiology (with Dr. Robert A. Good)

COUNCIL AND ADVISORY COMMITTEE MEMBERSHIPS (Past and Present)

International Transplantation Society, Council Member
Midwest Immunology Society
International Bone Marrow Transplant Advisory Committee

1977-78	Vice-chairman
1978-80	Chairman

National Foundation, March of Dimes, Clinical Research Committee
National Cancer Institute, Immunology Contract Committee

International Histocompatibility Testing Workshops, Councillor
National Institutes of Health, Experimental Immunology Study Section
Medical Science Review Committee, Juvenile Diabetes Foundation

PUBLICATIONS

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A serious participant of the workshop

Fritz Bach 1990

History of HLA: A Personalized View

Paul I. Terasaki

Address: UCLA Tissue Typing Laboratory
950 Veteran Avenue
Los Angeles, California 90024

Paul Terasaki

Microtechniques
1970

1990

ca. 1982

HISTORY OF HLA: A PERSONALIZED VIEW

PAUL I. TERASAKI

My interest in HLA stemmed from its possible application to transplantation. Though the implications of HLA were far reaching, far beyond applications to transplantation, one thesis I wish to develop here is that progress in HLA was fueled principally by its potential application to transplantation. Scientists do not live in a vacuum; they need funds to carry out their research, which is dictated by the social milieu at any given time. I would imagine that almost all grant requests for the study of HLA included some form of the statement that knowledge of HLA will contribute to society through the improvement of transplantation. The study of red cell types and gamma globulin allotypes has suffered by not achieving comparable funding levels and interest from the scientific community because they lacked comparable timely medical applications. In fact, with red cell types, Rh has received most of the attention, principally because of the consequence of immunization to the fetus. Other red cell polymorphisms will remain mere curiosities until a clinically relevant role is discovered for them.

Interest in clinical transplants began in 1955 when Hume, Murray and Merrill published their report of the first human kidney transplant of the modern era (1). The field started in earnest in 1963 when large numbers of transplants from living-related donors were performed in rapid succession. The sudden application to human transplants took the scientific community by surprise, for the scientific groundwork for such a monumental step simply had not been laid. Experimental skin graft survival, which was the standard model of the day, could not be prolonged beyond a few weeks. News of successful human kidney transplants took the field of histocompatibility testing out of the realm of theoretical examinations of mice. It was a widely held belief that tissue matching would be a significant component of uniformly successful transplants. The major funding source for tissue typing in the United States came from a special act of Congress in 1965, authorizing the creation of a Transplantation Branch within the National Institutes of Allergy and Infectious Diseases.

From a basic scientific view, pursuit of HLA knowledge was important in itself, and many were willing to accept funding, ostensibly in the name of transplantation. Even the name that we chose for ourselves, "histocompatibility", implied that the HLA antigens we were studying were part of a compatibility system in transplantation. Active associations today, such as the American Society of Histocompatibility and Immunogenetics, would collapse if tissue typing were no longer considered necessary for transplantation. Probably as much as 80% of meeting and workshop expenses are covered by transplant-related sources. Thus, even today, the field is being kept alive by funds from transplantation.

1958

The beginning of the HLA story might be placed in 1958, when Dausset first published a description of a leucocyte group he called Mac (2), for which he was awarded the Nobel Prize in 1980. I find it fascinating that Dausset did not have a follow-up paper on this finding until 1965, seven years later, when 4 other laboratories, independently, also identified the specificity.

Leading up to this discovery, Dausset had published a leukoagglutination method in 1952 (3). Others soon published on *variants of the leukoagglutination procedure: Miescher in 1954 (4); Brittingham and Chaplin in 1957 (5), and Killman in 1958.*

In 1957, Payne showed that antibodies to leukocytes occur in pregnant women (7), and independently, van Rood also detected leukoagglutinins in the sera of parous women (8). All these investigators were working in blood banks and were concerned with autoimmunity and the problem of transfusion reactions produced by leucocyte antibodies. The intense interest in this emerging field of leucocyte reactions is shown by Roy Walford's survey of 567 references by mid-1959 (9).

Nineteen fifty-eight was also a very fortuitous beginning for me. It was the year I received a National Cancer Institute Postdoctoral Fellowship to study under Professor Peter Medawar in the Department of Zoology at University College, London. Medawar, as everyone knows, was later knighted and received a Nobel Prize. My project was to identify the cell responsible for the graft versus host reaction in chick embryos (10). In the course of this work, I learned about handling blood cells and their identification with the phase contrast microscope. At that time, Peter Medawar and Leslie Brent were working furiously on cellular immunity to transplants, showing that the transplantation reaction was similar to the delayed hypersensitivity reactions produced by lymphocytes (11). It is difficult, even in retrospect, to understand why at that time I decided to take the opposite humoral immunity approach to transplantation.

Although my experience to that point had been limited to experiments with chickens and rabbits, I was anxious to get into studies with humans. I had read of Dausset's leukoagglutination work on humans with great interest. I asked my supervisor, Dr. Longmire, founding Chairman of the Department of Surgery at UCLA, for a year's extension of my study period so that I could go directly to Paris instead of returning to UCLA. He supported my plans enthusiastically, as he has done throughout my career at UCLA. So with my year's fellowship ended, I relocated my wife and two small boys from London to Paris. Perhaps it was because of my ineptitude in speaking French that after just one day, Dausset sent me off to the Pasteur Institute. Dausset and I have laughed about this incident on the many occasions we met over the next 20 years. Three months later, with no improvement in my language skills, and the impending birth of our third son, I decided to return to Los Angeles to work things out myself.

1959

Going back to the chicken, I tried to argue that in terms of being subject to fewer non-specific reactions, the lymphocyte agglutination test (12) would be superior to the then standard leukoagglutination test. While it had worked in chickens, subsequent experiments in mice showed that antibodies that agglutinate lymphocytes rarely occurred upon alloimmunization.

1960

FOURTH TISSUE HOMOTRANSPLANTATION CONFERENCE: NEW YORK ACADEMY OF SCIENCES: February 4-6, 1960, CHAIRMEN: JOHN CONVERSE, BLAIR ROGERS

Although this was the fourth meeting on transplantation, the field was obviously still in its early formative phases, since only 2 of the 46 papers presented dealt with transplants in humans. One described 3 patients who had parathyroid transplants, and the other, by Deally, Dammin, Murray and Merrill, described 2 patients receiving x-irradiation and a kidney transplant. One patient died after 25 days, but the other was still surviving one year after transplantation. Because of the complex radiation procedure with varying doses and the transfusion of bone marrow from 11 donors, Ferrebee *made the following comment in the discussion of the paper, "while the physicians may be somewhat confused regarding everything that happened, the patient recovered and went home." Was this to be the story of clinical transplantation?*

This was also the historic meeting at which Peter Gorer introduced the word "allograft" to replace the old "homograft".

Histocompatibility was discussed in terms of the number of genes and additive doses in skin grafts among teleosts, amphibia, hamsters, guinea pigs, and mice.

I was still interested in developing an in vitro test for compatibility, and therefore turned to the lymphocyte cytotoxicity as the better test to detect transplantation antigens (13). We showed that the reaction would be more sensitive if the antibody step was separated from the complement step (14). As source of complement, various combinations of sera from different species were compared (15) and we compared the use of rabbit serum to the then more widely used guinea pig complement (16).

1961

To resolve the argument that lymphocytes appeared to be the only suitable target cells, epidermal cells from rabbits were tested as target cells for tissue typing rabbits. Here we ran into the problem of autoantibodies to epidermal cells which in turn led to studies of autologous rabbit complement being used in the reaction (17).

1962

FIFTH TISSUE HOMOTRANSPLANTATION CONFERENCE: NEW YORK ACADEMY OF SCIENCES: February 8-10, 1962. CHAIRMEN: JOHN CONVERSE, BLAIR ROGERS

Good's group in 1959 (18) and Schwarz and Damashek in 1960 had shown that 6-mercaptopurine prolonged skin grafts in rabbits (19). The use of a derivative, (known as azathioprine and imuran) in dog kidney allografts was described at that meeting by Calne, Alexandre and Murray (20). Hamburger described 6 patients who received kidney transplants from related donors, 3 of whom died within 20 days and 3 had surviving grafts out to 2 1/2 years. Interest in cytotoxic antibodies was evident from the papers of the Mollers and of Boyse, Old and Stockert, who described cytotoxins against tumor allografts. The issue of the day was: is it the antibodies or is it the cells that kill off an allograft? Eichwald described the "white graft" phenomenon, which is rejection of the skin graft by humoral antibodies in parabiosis experiments. David Steinmuller showed the same rapid rejection of skin grafts upon passive transfer of immune serum to Lewis rat skin grafts produced in BN rats. Terasaki, et al, showed rapid damage to C57 mouse kidneys upon intrarenal artery injection of hyperimmune serum produced in A strain mice.

Having satisfied ourselves that antibodies reject allografts, we proceeded in earnest to try to use alloantibodies to detect the transplantation antigens. In order to have a more quantitative method, lymphocyte cytotoxicity was measured by Coulter counter (21). We realized from this, however, that even if the reaction could be measured quantitatively in 15 seconds, the method would be too slow to be applied to the clinical situation.

1963

CONFERENCE ON HUMAN KIDNEY TRANSPLANTATION: WASHINGTON D.C. September 26-27, 1963

According to the summary of the meeting provided by Dr. Joseph Murray, by this time, with the advent of azathioprine, as many as 244 human kidney transplants had been performed: 68 from cadaver donors, and the remaining from live-related donors.

From the abstracts, 11 had been done by Woodruff in Edinburgh, 20 by Hamburger in Paris, 13 by Goodwin at UCLA, 27 by Starzl in Denver, 8 by Calne in London, 16 by Kuss in Paris, 6 by Russell in Boston, 12 by Shackman in London, 67 by Murray and Merrill in Boston, 9 by Kolff and Nakamoto in Cleveland, and 20 by Hume in Richmond. Clinical renal transplantation was out of the purely experimental stage... and it was actually benefitting some patients. The race was on.

Finally in 1963, I was able to make the transition from animal to human studies. As an outsider in clinical circles, I had great difficulty obtaining blood samples to test. Unlike others already in the field who were affiliated with blood banks, I had no access to blood. Two co-authors on our first papers, Drs. Van de Water and Thomas Edgington, were residents who helped us obtain blood from pregnant women (22). Because of the scarcity of sera, whenever we obtained a tube of blood from a pregnant woman, we treasured the sample, were careful to aliquot the serum and scrupulously used the smallest possible amount for each test. From our work with mice we were accustomed to treating sera as valuable reagents. However, sera from humans were even more difficult to obtain.

Leukoagglutination gave many false positives, and the reproducibility of the tests was terrible. We tried all types of siliconization processes; use of plastic slides, etc. but nothing worked. Our 5 years of experimental work in animals helped us realize that the leukoagglutination test used by Dausset, Van Rood, and Payne was not precise enough. Granulocytes were simply too difficult to control, as shown by our earlier time- lapse cinematography studies (13). There was another feature of granulocytes that I didn't like; they died very rapidly in vitro, whereas lymphocytes were alive even on the second or third day after bleeding. Lymphocytes do not non-specifically agglutinate (12), but we were simply unable to find any human serum that would agglutinate lymphocytes. The lymphocyte cytotoxicity test, therefore, was the only other possibility.

No cytotoxic antibodies could be found in pregnancy sera when we used the then standard guinea pig complement. Turning to our previous studies of complements from various animals (15), we soon found that the rabbit complement we were using (16) and dog complement were effective. Dog complement was the stronger, but the thought of bleeding dogs for complement was something I did not have the stomach for. Dogs were available, since surgical experiments were being done daily in our Department of Surgery. We decided to stick to rabbits as our complement source, and our first paper describing results from the lymphocyte cytotoxicity test using rabbit complement was reported at the New York Academy of Science meeting, given below.

1964

SIXTH INTERNATIONAL TRANSPLANTATION CONFERENCE: NEW YORK ACADEMY OF SCIENCES, February 6-8, 1964; CHAIRMEN: JOHN CONVERSE, BLAIR ROGERS AND FELIX RAPAPORT

The principal clinical kidney transplant groups presented papers with figures similar to those given above in the National Academy of Sciences meeting held 4 months previously.

For tissue typing, this was an important initiation into the transplantation community. Van Rood presented evidence for his Four, Five and Six leucocyte groups, showing that compatibility for these groups led to skin graft survival of 13.4 days, compared to survival of 11 days if these groups were incompatible. Bach and Hirschorn showed that the mixed lymphocyte culture reaction measured incompatibility between different individuals. Colombani and Dausset tested 253 individuals with 48 leukoagglutinating

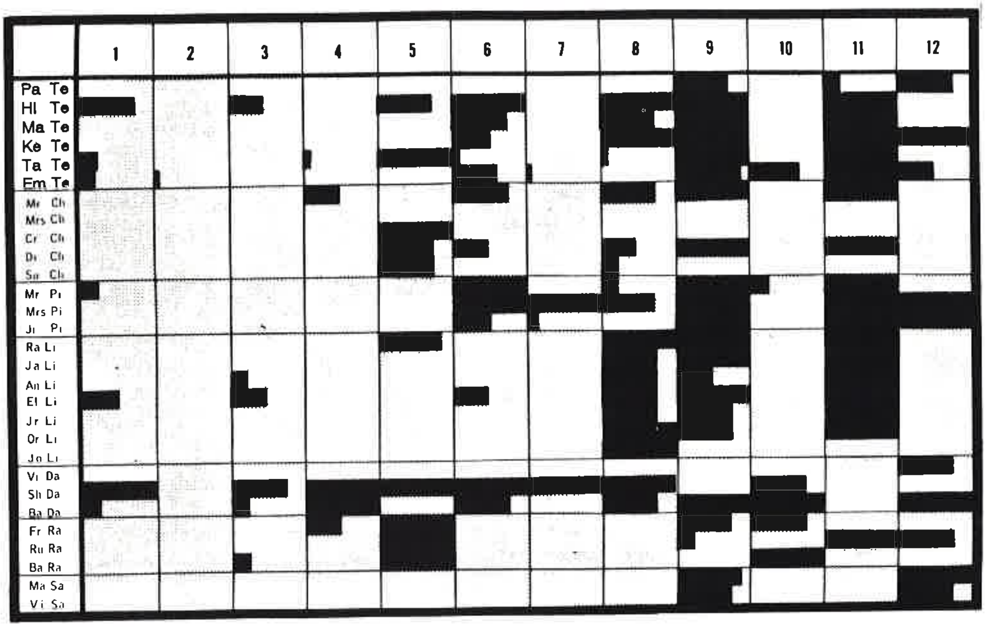


Figure 1. The lymphocyte cytotoxicity reactions of 6 families are shown with the father, mother and children listed in that order. The length of the bars indicates the strength of the reactions. The Terasaki family is the first one listed. 1964 (22)

sera and 18 persons received skin grafts from one most compatible and one most incompatible donor. Results showed some possible relationship between graft survival times and leukocyte compatibility. There was no mention in that paper of the Mac group. The sera were used individually, and not as defining any particular group.

Ceppellini described a program of planned immunization with blood transfusions to produce typing sera. "The majority of the recipients are chronic psychiatric patients secluded in a local asylum. They have been selected.. because of a state of anemia or hypoproteinemia, a common phenomenon in individuals with difficulty in feeding or subjected to protracted therapy with psychotropic drugs." Eleven recipients were given weekly transfusions (80 ml) for as many as 13 weeks. Interestingly, 8 did not form any leukoagglutinating antibodies after receiving 1,200 ml of blood over the course of a year. A second group of 23 recipients received 6 transfusions, and 3 had produced antibodies. The main questions dealt with the reproducibility of the leukoagglutination tests, and monospecificity of the antisera. Terasaki reported on a semi-micro lymphocytoxicity test which used 0.01 ml of antisera and rabbit complement. The test was done on a slide, and cells were examined by nigrosin dye exclusion and phase contrast. Lymphocytes from 32 random individuals and a similar number of related individuals were tested with a panel of 12 antisera, and the reaction patterns were given (Fig. 1).

From this group of papers, one gets the feeling that now the competition for the tissue typing resolution was serious.

After we mentioned the importance of rabbit complement to Roy Walford, who also worked at the UCLA Medical Center, he tested many complement sources, and agreed that rabbits were the best source of complement (23). In Figure 1, our serum 1, 3, and 8 which react similarly, defined our group 2, which was later shown at the 1965 Leiden Workshop to be the HLA 2 or the Mac specificity of Dausset. As seen in the figure, I am negative for this group and my wife is positive. The reaction of my daughter with serum 10 when both parents were negative seemed to be a matter of concern, since the reaction was rechecked according to the paper. This was the first recorded paternity test done by lymphocyte cytotoxicity. It was to take some 10 years before such false reactions could be eliminated and the test could be used reliably in the courts for paternity testing.

Although I had bled all types of animals, I was reluctant to learn how to bleed people, as was my technician, John McClelland. So we wound up finger poking each other to obtain blood for each day's experiments. The blood was taken in heparinized capillary tubes, the buffy coat cut off with precision, put in a petri dish, and a coverglass applied. The granulocytes stuck to the dish upon incubation. Lymphocytes were then washed off, and the remaining red cells lysed (24). The number of cells we had for testing, as well as the serum supply, was very limited. In no instance did we have antibodies other than from the original 5-10 ml tube of blood. We were simply unable to get a rebleeding or larger quantities of blood. We were definitely far behind the competition who were already skin grafting and transfusing volunteers. I had difficulty enlisting my 3 little sons as volunteers for finger pokes and was sneaking around, heel sticking my sleeping newborn daughter.

These were the circumstances under which the microdroplet test was born. It was apparent that to get any further, even 0.01 ml per test, or 10 lambda per test was just too much. We had to aim for a test that used only 0.001 ml. I noticed a very significant feature about tests being done at that time. After a test was run in tubes, only a small fraction of the cells were taken out to look at the reaction under the microscope. Consequently, all the other cells were being tested unnecessarily. So by doing the test on the slide in the first place, we solved the first part of the problem. But even with the test on a slide, we wound up looking through the microscope at only a very limited random spot on the slide. What we needed was a system whereby the reaction consisted ONLY of the cells that we eventually looked at in the microscope.

Four years previously, in 1960, I was invited to lecture at Stanford, where I met Gus Nossal. I still remember his statement, "every new advance in science is the result of a technical methodology breakthrough." At that time he was referring to the excitement that surrounded the work of single cell cultures in studies of antibody production. He was using the de Fonbrune oil chambers in which micromanipulators place the hanging drops within the chamber. It was 4 years later when I recalled having seen what was necessary to solve our problems. Instead of using the conventional upright microscope, we decided to convert to the inverted microscope, and convert the de Fonbrune chamber into an oil DISH that could be accessed from the top. We could investigate many droplets by looking at the cells from underneath, thereby eliminating the cumbersome micromanipulation equipment. The oil, however, was absolutely essential, since such small quantities would immediately evaporate.

We could not use the conventional slides to look at the cells from underneath because they were too thick. We soon became one of the biggest customers for the large 3"x4" coverglasses. An oil chamber had to be constructed with the coverglass bottom. First we used scotch tape and frames formed from aluminum foil (24). I was determined not to use the tedious micropipettes for dispensing the small quantities, so we experimented with the conversion from pipettes to microsyringes manufactured by the Hamilton Company in Los Angeles. In the original description, the 3 critical elements listed for the microtest were: 1) the inverted phase contrast microscope, 2) the micro syringe clicker which dispenses one lambda (0.00l ml) per click, and 3) the oil chamber. They still remain the basic features of every tissue typing laboratory today.

FIRST INTERNATIONAL CONFERENCE ON HISTOCOMPATIBILITY TESTING: NATIONAL ACADEMY OF SCIENCES, WASHINGTON D.C., CHAIRMAN: PAUL RUSSELL, June 7, 8, 1964. HISTOCOMPATIBILITY WORKSHOP: June 9-12, CHAIRMAN: BERNARD AMOS

In their introductory remarks, Paul Russell and Leslie Brent expressed their hopes that one day, some form of tissue typing would be developed to select the best donors for transplantation. By platelet complement fixation techniques, Shulman described several specificities. The group he called PlGrLy B1, which reacted with 46% of the panel, was especially interesting and was shown at the next Workshop to be the same as the Mac or HLA 2 specificity. Van Rood provided extensive data for 3 groups of allelic specificities, Four, Five and Six, together with family studies. Payne showed data on grouping sera into 2 allelic groups; LA 1 and LA2 (later renamed HLA A1 and HLA A2). This data was also given by Bodmer at the simultaneously held Cold Spring Harbor Symposium. Dausset described a group Xa and Xb which he thought might correlate with van Rood's 4a and 4b. Using these groups, and 40 other leukoagglutinating sera of unknown specificity, selection of donors and recipients resulted in differences in skin graft survival of almost 2 days. Again, no mention of the Mac specificity was made.

Metzgar, Zmijewski and Amos showed that leukoagglutinins from parous women were often weak and contained complex mixtures of antibodies. Antibodies made in 7 of 9 subjects immunized with lymphocyte suspension had moderate titer, and also contained a mixture of antibodies. Antibodies produced in chimpanzees were made monospecific by absorption techniques. Ceppellini reported on his prize sera, Buffo, produced by planned immunization. During a 2-year period of immunizations, the titers declined rapidly, but responded well to boosting. The production of monospecific leukoagglutinating sera was sought by extensive absorption experiments. In a discussion, Walford described finding lymphocyte cytotoxic antibodies after skin grafting. Mixed leucocyte tests for compatibility testing were described by Bain, Lowenstein, and Maclean and by Bach and Hirschorn. Gray and Russell described a similar in vivo MLC or a normal lymphocyte transfer test in which the reaction produced in the skin by lymphocytes was taken as a measure of donor-recipient compatibility. Carpenter and Merrill commented on similar experiments.

Terasaki presented the first paper describing the testing of kidney transplant patients with the lymphocytotoxicity test together with the first description of hyperacute rejection. Twelve of Dr. Starzl's kidney transplant patients (Denver) and 2 of Dr. Goodwin's patients (UCLA) were tested with a panel of 55 cytotoxic antisera. From the retrospective finding of preformed cytotoxic antibodies against the donor in a brother-to-sister transplant that never functioned, it was suggested that a crossmatch test be done before kidney transplants to avoid immediate rejection.

The workshop at Duke consisted of demonstrating the various methods in existence at that time. The principal method was leukoagglutination (Dausset, Amos, Van Rood, Payne, Lalezari) followed by mixed cell agglutination (Milgrom, Metzgar), complement fixation (Wilson), indirect antiglobulin consumption (Colombani), mixed *leucocyte culture (Bain, Lowenstein, Hirschorn) and micro lymphocyte cytotoxicity (Terasaki).*

Although Dausset, van Rood, Amos, and Ceppellini were feverishly working on skin grafts to show the relationship of the skin rejection time with the serologic reactions, I was determined not to get into that competition. It did not seem reasonable that kidney transplant surgeons would be convinced about the value to tissue typing based on a few days difference in skin graft survival times. I was lucky enough to meet Tom Starzl, who enthusiastically supported our studies on tissue typing his patients. We no longer had to go out begging for samples to test, and within a few months we had tested enough patients to write a paper. These tests on blood sent from Denver would not have been possible with the leukoagglutination test, which must be done on fresh blood. Because most of the patients were heavily immunosuppressed, the number of obtainable lymphocytes was small. Without conversion to the microtest at just that time, the studies could not have been carried out.

1965

SECOND INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: Leiden, August 15-21, 1965; CHAIRMAN: JON VAN ROOD

Under the leadership of Jon van Rood, the task at hand was to test antisera against a common panel of cells to see if the specificities being defined in the different laboratories correlated with those in other laboratories. Fourteen groups of investigators brought a total of 118 antisera to be tested on a common panel of 45 blood donors. From the analysis of the workshop results, 6 laboratories now had defined groups. In the Workshop, 14 van Rood groups were tested, 4 Shulman, 2 Payne, 8 Dausset, 2 Amos, and 6 Terasaki. Most striking was the strong correlation found for Mac (Dausset), B1 (Shulman), LA2 (Payne and Bodmer), group 2 (Terasaki), and 8a (Van Rood). This was the first demonstration that the HLA 2 specificity did indeed exist, and that many antisera to it could be found independently. The same specificity had been detected by leukoagglutination, platelet complement fixation, and lymphocyte cytotoxicity.

As for the methods used for typing, the tide was beginning to turn. Although leukoagglutination tests were used by Ceppellini, Dausset, Lawler, Nelken, van Rood, Wilson, Zmijewski, Jensen, Kissmeyer-Nielsen and Payne; the cytotoxicity test was now used by Amos, Batchelor, Ceppellini, Engelfriet, Ivanyi-Dausset, Terasaki, and Walford. The microtest, however, was used only by our lab and by Ceppellini, who was the first to see the merits of the test. Shulman did the complement fixation on platelets and the platelet agglutination test was done by van der Weerdt.

Dausset and Ivanyi made a major advance in detecting 10 specificities by switching to the lympocytotoxicity test. They examined the 10 antigens in French, Japanese, and Malayan populations. From the data, Dausset concluded that one genetic system was involved, which he called the Hu-1 system. However, from the evidence given, one could not determine conclusively that a single locus was involved. Van Rood presented evidence for 5 new leukocyte antigens. The normal lymphocyte transfer test was shown to correlate with the presence or absence of leukocyte compatibility. From skin graft experiments, the 6c and 6d antigens were thought to be strong transplantation antigens. Ceppellini also considered the possibility of a single locus from analysis of kidney transplant data and from his skin graft experiments. The skin graft survival in siblings was 15.2 days, parents 14.2 and unrelated 11.9 days. In the same year, Simonsen also proposed that the human kidney transplant data could *be interpreted to show a single locus with relatively few alleles (25).*

The production of many interesting antisera by skin grafting was given by Walford. Bodmer described 2 new groups which he called 4c and 4d. Amos described 3 groups with the skin grafting of 16 subjects based on which might or might not be compatible with a panel of sera. Terasaki and McClelland described the first freezing of lymphocytes for typing.

Vredevoe, Terasaki, Mickey, Glassock, Merrill and Murray reported results of correlation with kidney graft survival. Fifteen kidney transplant donor and recipient pairs from the Peter Bent Brigham Hospital were examined. Compatibility for 6 specificities was examined, as well as compatibility for a panel of 100 antisera. Although it was expected that all these patients who had survived for 6 months or more would be well matched, it was noted that many incompatibilities existed in these kidney transplant survivors.

This study of the patients from Boston's Peter Bent Brigham Hospital was done with the help of Dr. Richard Glassock, who after completing his residency at UCLA, went to work with Dr. Merrill. Glassock (now Chairman of Medicine at Harbor UCLA Hospital) was the liaison between the two groups. The first author of that paper, Dr. Donna Vredevoe (now Associate Dean of the UCLA School of Nursing), was the first postdoctoral fellow at our lab. The other author was Dr. M.R. Mickey, a vital part of everything we did in our lab for 25 years, until his retirement in 1989. He was principally responsible for the most difficult aspects of our work: the classification of antisera into the specificities and the analysis of kidney transplant results.

1966

SEVENTH INTERNATIONAL TRANSPLANTATION CONFERENCE: NEW YORK ACADEMY OF SCIENCES: February 14-16, 1966; CHAIRMEN: JOHN CONVERSE, FELIX RAPAPORT, AND JEAN DAUSSET

Interest in antilymphocytic serum was intensifying with papers by Levey and Medawar, and by Monaco and Russell. Starzl reported on his classical series of 64 patients treated between 1962 and 1964 in which the one-year graft survival was 33% for living unrelated donors, and 67% from live-related donors. Obviously, the genetic matching between the donor and the recipient was important.

Russell reported on the correlation of the normal lymphocyte transfer test with the clinical outcome of 12 kidney transplant patients. Dausset provided an excellent review of the current state of agreement in specificities among the tissue typing laboratories and reiterated his view that there was only a single system. The major argument revolved around positive and negative correlations between all the sera. Although this relationship had been confirmed, the existence within the whole system of 2 allelic groups, the A and B locus, had not yet been recognized. Based on 95 test skin grafts, Ceppellini discussed the probable genetic parallel between kidney graft survival and the short survival of skin grafts. Van Rood provided a summary of the Leiden Workshop, as well as a correlation between skin graft survival and compatibility for his leukocyte groups. Bodmer described a new allele, LA3 (which later became HLA 3), and the concept that known specificities fit best into 2 independent systems: the LA and 4a,b,c,d systems. From subsequent developments, and the studies of Kissmeyer-Nielsen and Svejgaard, this grouping was later shown to be the HLA A and B loci.

The first application of typing to selection of kidney transplant donors was described by Terasaki, Starzl, et al. There was some improvement in transplant results in 18 grafts performed from live-related donors and 14 patients who received grafts from unrelated donors, though the experience was relatively recent. Vredevoe, et al showed that sera from many typing laboratories, tested by the micro lymphocyte cytotoxicity test, fit into defined group clusters. Batchelor described 17 sera produced by skin grafts and their absorption to produce group specific antisera. Balner and van Rood immunized Rhesus monkeys, and applied serologic testing paralleling that of the human experience. Zmijewski and Amos compared results from the agglutination and cytotoxicity tests.

Caught up by Starzl's enthusiasm, we began tissue typing many subjects on a prospective basis. Most interesting was the typing of prisoners. The choices were usually from a donor pool of about 30 prisoners, resulting in the transplantation of 18 patients. Soon after this experience, by international agreement, all use of prisoners was discontinued.

The need to type ever increasing numbers of donors and patients placed great stress on our laboratory. It was obvious that the oil chamber had to be improved since leaks developed at the scotch tape seal and oil was everywhere in the lab. We drew up plans for a plastic tray with small wells and a thin coverglass thickness bottom. Although I was able to persuade Falcon, a local plastics company, to make the tray, I was unable to convince the University Patent Office that the tray would be worth patenting. They concluded that the tray would never sell enough to recover patenting costs. Still, it was an agonizing 2 years before the plastic tray was finally produced commercially. Figure 2 shows the various trays we used in the interim. By 1966, we were desperate. The plastic trays were finally introduced at the Third International Histocompatibility Workshop, marking the point at which the test finally became readily exportable to any laboratory.

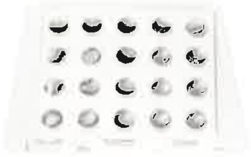
In our first steps towards automating the test, we attached an electric typewriter to the microscope so that by pushing a button, the score could be recorded without taking one's eye from the scope and without having to pick up a pen to record every well. The solenoids used to pull down the keys on the electric typewriter were round and fat and could only be used for every other number key, that is, 2, 4, 6, and 8. This accounts for the rather bizarre 2,4,6,8 code used today by practically all tissue typing laboratories for recording their results.

1967

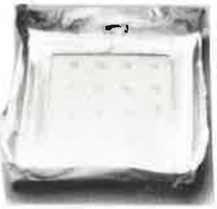
CONFERENCE ON TRANSPLANTATION: SANTA BARBARA, January 11-13, 1967; CHAIRMAN: WILLIAM LONGMIRE

Essentially all the early and later day leaders of the field could be found among the 200 participants of that meeting. Murray and Barnes reported that in the Registry, 1084 patients had received kidney grafts, 40% of which were from cadaver donors. Over 20% of the kidneys from cadaver donors never functioned, compared to less than 10% from live-related donors that never functioned. Obviously, crossmatching was still not widely practiced. Starzl, et al reported their second series of 42 transplants in which related donors yielded 66% one-year graft survival and unrelated donors 55%. They spoke of reaching an impasse, since their second series was not much better than their first series of 64 patients. Williams and Hume reviewed 101 kidney transplants, reporting an interesting figure of 100% success rate if there was no rejection, and a progressive falling off of graft survival rates in groups with 1, 2, or 3 rejections. The principal authors of other reports of kidney grafts were: Dossetor, 59;

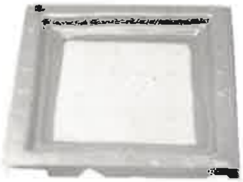
Evolution of the Microtest Tray



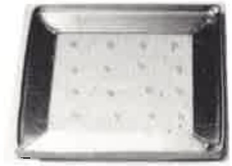
1959 0.01 ml test, 10 lambda



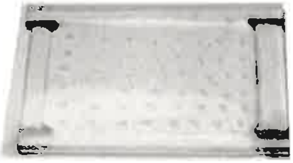
1/64-5/64 First 0.001 ml, one lambda microtest, aluminum foil oil chamber



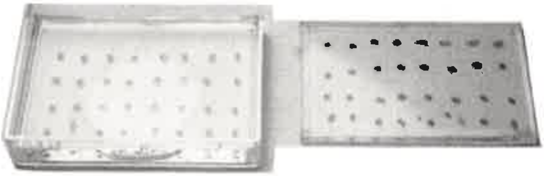
5/64-7/64 Toy vacuform plastic frame oil chamber



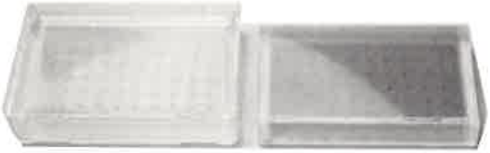
7/64-9/64 Stainless steel frame oil chamber



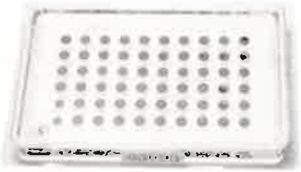
9/64-5/65 Glass frame with PVP glue



5/65-11/66 Oil tray, remove coverglass to read with lucite frame



11/66 to 6/67 Wells made from wax-beeswax mixture, oil chamber for reading



6/67 to??
Injection molded
styrene tray
oil reduced to
0.005 ml per well

Figure 2. The standard microtest is often called the NIH test, but this is only because a meeting was held at the NIH at which the incubation times were set as a standard.

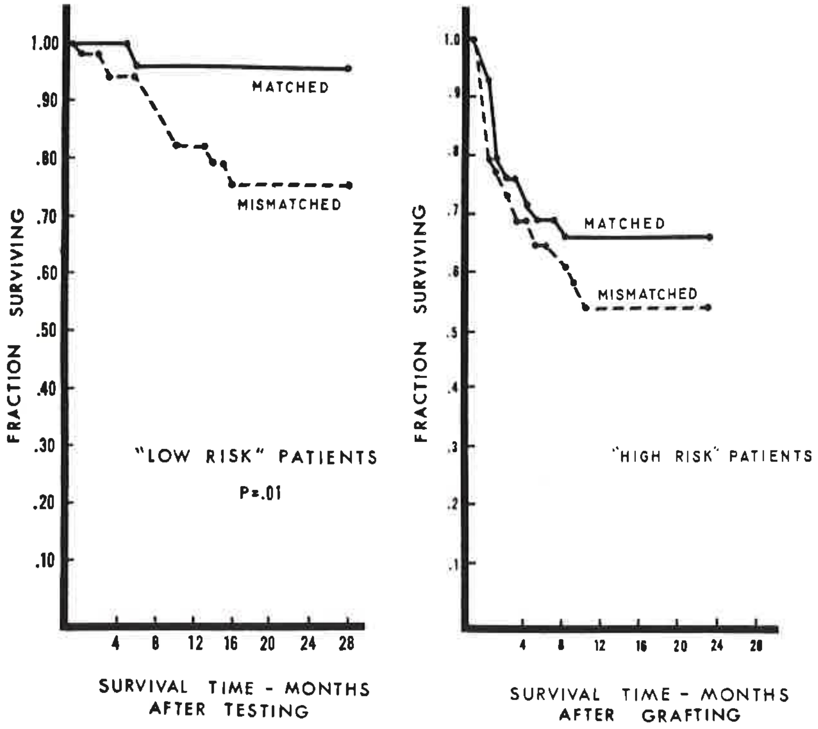


Figure 3. In this retrospective study, the patients were divided according to when they were typed in relation to their transplant date. Those typed up to 3 months posttransplant were classified as low risk and those typed after the initital 3 months posttransplant were considered high risk. 1967 (Transpl 5:1057)

Nakamoto, Straffon and Kolff, 79; and Hamburger, 67.

Amos and Zmijewski reported testing 7 kidney transplant patients at Duke. Terasaki, et al reported on the survival of 196 kidney transplant patients classified according to matching for 7 specificities. Blood from the donors and recipients had been received from Denver (Starzl), Paris (Hamburger), Richmond (Hume), Cleveland (Kolff), San Francisco (Najarian) and UCLA (Goodwin). The patients were divided into high and low risk groups based on whether they were typed within the first 3 months posttransplant or after the initial 3 months posttransplant (Fig. 3). The most striking feature commented upon was the high survival of the mismatched patients. The difference between matched and mismatched was not as great as had been expected.

Faced with the necessity of receiving blood from so many centers, we had to devise better shipping methods. I recall that it was most frustrating to receive blood from Paris. No matter what we wrote on the package... "LIFE OR DEATH", "URGENT", before clearing them, the customs people would always hold them for several days. We handled that obstacle by developing a flat plastic bag that would fit into an ordinary air mail envelope. The bag was filled with glass beads that acted similar to sand soaking up water. The blood-soaked beads did not leak and the airmail envelopes marked simply "SPECIAL DELIVERY" were received within 2 days without customs inspection. We subsequently improved the transport system with the use of sanitary tampons enclosed in a plastic bag that soaked up blood better than glass beads. In this way, the early kidney transplant patients at the Necker Hospital in Paris were first typed in Los Angeles.

The relationship between HLA matching and transplant outcome shown by the first survival curves of pooled transplant results in Figure 3, has not changed noticeably in the subsequent 20 years. Mismatched grafts survive well, and the difference with matched transplants is 10-20% at one year.

THIRD INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: Torino, June 14-24, 1967; CHAIRMAN: RUGERRO CEPPELLINI

It was at this important Workshop that the concept of a single locus with two subloci was finally proven. After the conference, a WHO nomenclature meeting was held and the name HLA was designated for the locus. The Workshop was masterfully organized and taken to its conclusion by Ceppellini. His chapter in that book is the classical study which established for all time the single HLA locus. Eleven families and 21 unrelated donors comprised the workshop panel that was tested by the following 16 teams using 476 antisera: Amiel, Amos, Balner, Batchelor, Bodmer, Ceppellini, Dausset, Engelfreit, Lalezari, Lawler, Kissmeyer, Payne, Terasaki, van Rood, Walford and Zmijewski. As many as 13 specificities were then detected independently by different laboratories. Most impressive was the way in which essentially all the complex serologic reactions within the families could be assigned to the 4 haplotypes, showing clearly that only a single locus was involved. The MLC test described by Bach also could best be explained by a single locus.

Using antisera raised by skin grafting, Walford emphasized the concept of monospecificity as shown by extensive absorptions. Van Rood reported studies on 54 kidney transplant patients tested by leukoagglutination. Similarly, Payne noted that among the 25 recipients tested by leukoagglutination, 5 of 9 mismatched recipients still had good function 19-36 months after transplantation. From the 209 patients tested by micro lymphocyte cytotoxicity, Terasaki concluded that despite incompatibilities in the 6 groups, many patients were surviving with good clinical function (Fig. 4). It was at this meeting that van Rood proposed the formation of Eurotransplant.

Bodmer introduced the use of fluorochromasia, previously used by others (26) for the purpose of showing the living cells in the microtest. Bruning took later this development to its conclusion with the addition of ethidium and propidium as the dye for dead cells. The fluorescent microtests of today are based on the various refinements made by Bruning, such as the use of India ink and hemoglobin for quenching (27).

Amiel stimulated interest in correlation of the leukocyte antigens with disease by showing that 4c (HLA B5) was correlated with Hodgkins disease. It is interesting to note that despite triggering 53 subsequent studies representing more than 2000 patients, this "classical" study, often cited as the first demonstration of an association of HLA with disease, still remains unconvincing.

Our tissue typing team from UCLA consisted of M.R. Mickey, John McClelland (who is still with us, 33 years after starting in our UCLA Tissue Typing Laboratory), Peter Morris, and Marietta von Diepow, a tech-

Correlation of Clinical Ranks with Incompatibility for Leucocyte Groups Determined by Posterior Probability Analysis

Mismatched Group	Clinical Rank and Score					Mean Clinical Rank Score	t ratio	P
	A	B	C	D	F			
0	52	21	11	3	9	.57		
1	1	6	5	1	3	.34	3.43	<0.001
2	4	1	2	1	3	.41	1.74	0.04
4	8	1	2	2	1	.55	0.18	0.45
5	9	4	1	0	7	.46	1.51	0.07
6	1	2	3	1	3	.30	3.11	<0.001
Two or more groups	10	7	3	4	4	.46	1.91	0.03
One or more Groups	33	21	16	9	21	.43	3.54	<0.0005

Figure 4. Transplant patients were classified according to the mismatch found for the 5 specificities examined. Clinical ranking of the patients was from A, the best down to F for failure. Note the mismatched patients with good clinical ranks. 1967 (Third Workshop)

nician. Marietta was stolen, or should I say captivated and captured by Dr. Domenico Bernoco, whom Ceppellini had sent to our laboratory to study. They were married and worked in Ceppellini's laboratory at Basel. After about 2 years, the tables were turned when Marietta became homesick for Los Angeles, and persuaded her husband Bernoco (now a Professor at U.C. Davis) to come back to our laboratory. I would have loved to hear if Ceppellini had ever forgiven me for this turn of events.

On another interesting historical note, work done in our lab by another member of our team, Peter Morris, showed that lymphocytotoxic antibodies were produced after transplant rejection (28). Probably known to all is Morris' subsequent important work in histocompatibility and transplantation and his appointment as the Professor of Surgery at Oxford.

FIRST INTERNATIONAL CONGRESS OF THE TRANSPLANTATION SOCIETY: PARIS, June 27-30, 1967; CHAIRMEN: JEAN DAUSSET, JEAN HAMBURGER, GEORGES MATHE

Bernard Amos was involved in the formation of the International Transplant Society and became the past president, while Sir Peter Medawar was elected the first president. In the genetics of transplantation session, Ceppellini gave a report of the just concluded Third Histocompatibility Workshop. Amos examined skin graft survival among siblings in 4 families. Those siblings who were identical for HLA had a 10 day longer survival time than those who differed by 1 or 2 alleles. Van Rood reported on the retrospective matching of 65 kidney transplant patients from living donors gathered from 8 different transplant centers using the leukoagglutination test. Transplants from siblings who were compatible had better survival than other incompatible pairs. Payne, Perkins and Najarian showed that in 22 kidney transplant patients, the matched pairs had superior outcome when typed for 7 specificities by leukoagglutination. Terasaki showed that among 218 patients, as many as 23% of males and 46% of females had preformed antibodies. Such antibodies were found in 7 patients who suffered immediate rejection, and in every instance, the antibodies were *cytotoxic to the transplant donor. Six of the 7 patients were females, and all had been pregnant. Kissmeyer-Nielsen showed that 7 of 30 patients had leukoagglutinins and 4 had lymphocytotoxic antibodies. Two patients with leukoagglutinating reactivity against the donor suffered hyperacute rejection (29). Dausset provided data on the HLA system supporting the concept of a single locus with 3 main series of antigens: the "1" ,"3" and "7" series. Walford discussed the prospects for donor selection by MLC and serology.*

1968

WHO NOMENCLATURE MEETING: NEW YORK, September, 1968

This was the critical meeting at which the HLA name and first specificities were agreed upon. Fortunately, Dr. Walford tape-recorded the meeting, which appears verbatim in his chapter. It offers a vivid demonstration of how the agreement was hammered out and it makes all the personalities involved identifiable.

By this time with 4 years of experience with the microtest, Dr. Kamal Mittal of our laboratory systematically examined the various ways of making the test more sensitive: the time necessary with antibody and with complement; incubation with shaking; hypotonicity of complement; addition of complement after shaking out of antibody (later to be popularized as the flick method); use of Coombs reagent (later antiglobulin); trypsinization of the lymphocytes; use of trypan blue; and use of lyophilized serum trays (30). Surgeons were not convinced of the value of crossmatching until Dr. Ramon Patel, in our laboratory, provided evidence showing that in patients with a retrospective positive crossmatch, 24 of 30 had immediate failure (31). Among patients with a negative crossmatch, 8 of 195 had immediate non-function. With this evidence, crossmatches became a routine prospective test for all kidney transplants.

The first evidence that tissue typing was effective in selecting the HLA identical sibling donors for kidney transplants was provided from our laboratory by Dr. Dharham Singal (now Professor at McMaster University) (32). The clear evidence showing the difference between sibling donor and parental donor transplants is given in Figure 5.

Now more centers wanted to open their own tissue typing laboratories and the microtest permitted them to share reagents. This was facilitated by shipping tissue typing trays pre-filled with the key reagents. The microtray required a total of only 0.06 ml of reagents for testing 60 different reagents, making it an ideal way for many laboratories to share scarce reagents. New laboratories needed only to prepare the lymphocytes, add them to the tray, and read the reactions. From 1968, under a contract from the National Institute of Allergy and Immunology, we supplied tissue typing trays to over 50 laboratories for typing transplant patients. In this way, transplant donors and recipients were typed on a uniform set of reagents throughout the United States and Canada.

1969

It was now apparent to us that if tissue typing were actually to be used for human kidney transplants, a method by which the kidneys could be shipped would be necessary since in any given area there would not be sufficient numbers of patients waiting to attain good matches. In 1969, the Belzer machine was the main method by which kidneys were stored (33). We initially worked with Dr. Donald Martin and Dr. Ian Grey to devise a simpler cold preservation method. We were fortunate in having Dr. Goeffrey Collins come to our laboratory after finishing his Surgical residency in England. Collins showed that hypothermia alone was sufficient for preservation of dog

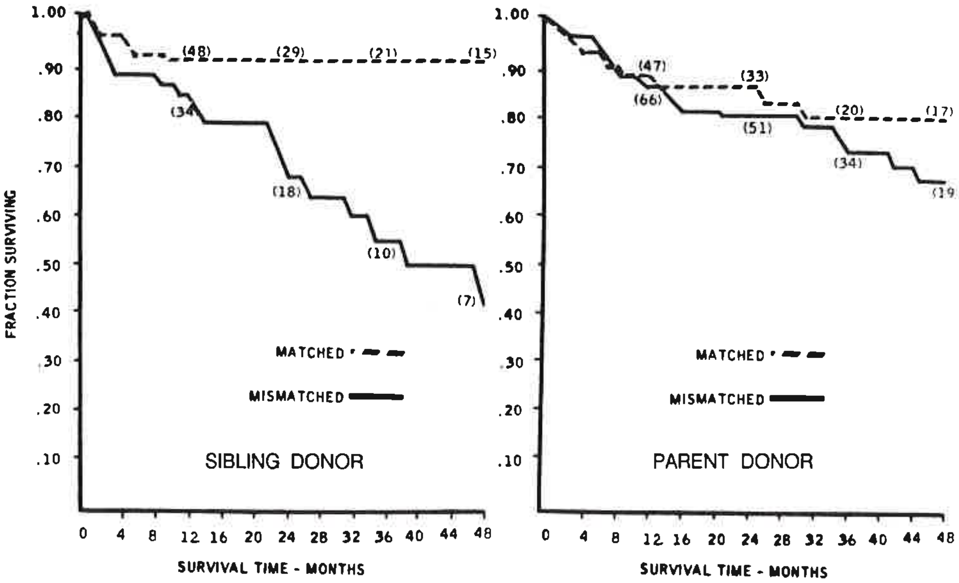


Figure 5. HLA matched kidney transplants from sibling donors are shown for the first time to have far superior survival to those who were mismatched. HLA was also shown to be ineffective in separating kidneys from parental donors. 1968 (32)

kidneys stored for 12 hours (34), but for 30 hours, a new solution, with more intracellular composition was necessary (35). To show others that this solution was effective even for international kidney exchanges, dog kidneys were shipped by ordinary commercial airlines from Los Angeles to London, Tel Aviv and Sydney. Then Collins implanted them into British, Israeli and Australian dogs as he went around the world (36). It still required many years to have the solution's effectiveness finally accepted, but today, over 90% of all cadaver donor kidneys throughout the world are preserved with this solution or variants.

1970

FOURTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP, January 20-29, 1970; CHAIRMAN: PAUL TERASAKI

This was the first workshop to start an exchange of antisera by mail and to use the same standardized micro lymphocytotoxicity test by mutual agreement. One hundred-sixteen antisera were selected from 21 laboratories for the Workshop, and 298 families were tested with these sera by 17 laboratories. The results were entered into a computer and analyzed by Mickey. From this massive cooperative project, the HLA specificities HLA 1 to HLA 13 were first established. Moreover, other HLA specificities up to HLA B27 were identified provisionally, and given a WHO designation. Thus, as many as 27 specificities were defined for the first time. Final proof of the single chromosome with 2 sub-loci was obtained from the family studies.

The conference provided many new findings which served as the nidus for future development. Sandberg, Thorsby and Kissmeyer-Nielsen provided the first *evidence of a third C locus. Mottironi and Terasaki reported on the existence of cold lymphocytotoxins as autoimmune antibodies. First isolations of soluble HLA antigens were reported by Reisfeld, et al and Mann, et al. Soluble HLA antigens in the serum were found by Van Rood. Bates and Sell described the first instance of a frozen lymphocyte tray to be used for screening antisera. Thorsby and Bratlie introduced Boyum's method of ficoll hypaque for isolation of lymphocytes to the HLA community (37). One of the exciting aspects of the meeting was the possible replacement of the micro lymphocyte cytotoxicity by a micro complement fixation reaction, as presented in papers from van Rood, Bodmer, Walford and Colombani-Dausset. The principal advantage was supposed to be that the results could be read by eye, without a microscope. Following an extensive serum exchange at the 1975 Workshop, the test was abandoned.*

The six subsequent workshops all followed the plan set by this one: exchange of sera by mail, use of the standard micro lymphocyte cytotoxicity test, and a workshop meeting followed by a conference and WHO nomenclature meeting.

Dr. Ekkehard Albert, who had come to our laboratory as a post-doctoral fellow, played a critical role in digesting, sorting and summarizing the results of the complex serologic reactions. He subsequently became the Chairman himself of an International Histocompatibility Workshop 14 years later. Dr. Miguel Kreisler, another postdoctoral fellow in our lab at that time (now a Professor in Madrid), analyzed the HLA matching correlation with transplantation, but because he could not get a good association, decided not to publish. This led him to the first studies on presensitization (42).

The joint decision made by the tissue typing group to use a single test with a standard set of incubation times was crucial to the subsequent development of the field. Without it, new workers couldn't enter the field and international collaboration, so vital for working out the fine points in specificity definitions, could not have taken place. The microtest allowed the exchange of small volumes of the precious reagents. Since most laboratories were using sera from pregnant women, it was difficult to obtain large quantities for local use as well as for exchanges.

THIRD INTERNATIONAL TRANSPLANT SOCIETY CONGRESS; HAGUE, September 7-11, 1970; CHAIRMEN: HANS BALNER AND D. W. VAN BEKKUM

This congress is remembered as one of the more important meetings, with many interesting advances and a superb social program. The rapid growth of the field can be seen by the 1600 page report of the meeting proceedings, a review of which will not be attempted here. However, one of the memorable papers of the meeting sent tissue typers and surgeons on divergent courses for the ensuing 20 years. The paper underscored the lack of correlation between HLA matching with the results of cadaver donor transplants and drew attention because by 1970, tissue typing had reached its pinnacle. Everyone believed that patients who failed were those who were mismatched, and those who succeeded must have been matched. The data from the HLA identical siblings was now accepted by all, and it seemed obvious that the results should be readily transferred to unrelated cadaver donors. A European organ sharing organization, "Eurotransplant", was created in 1968 by van Rood, and by 1970, it was operating in full swing. The name has always been somewhat of a misnomer since Scandiatransplant and France Transplant were established shortly thereafter, and in addition today we have U.K. Transplant, Italy Transplant, and Swiss Transplant. In the

United States, we also had started a citywide sharing scheme in November, 1967 based on matching (38), and a national pool of recipients (39). Though objections from surgeons are common today, in 1970 they were ready to accept HLA typing as a means of kidney selection for transplantation. The heretical presentation based on the largest series of patients of the day was completely unacceptable to tissue typers.

Interestingly, this paper does not appear in the proceedings of the meeting. It raised such a storm that it was reassessed and submitted later by M.R. Mickey for publication in Tissue Antigens (40). One of the key tables from that paper is reproduced here as Figure 6. Sibling donor grafts correlated well, but for the 147 cadaver donor kidney transplants tested with 11 HLA specificities, there was no statistically significant association. The marked similarity to data published 3 years previously (Fig. 4) can be noted. The principal problem remained the same over the years: many mismatched patients had good survival.

Since our data on the correlation of cadaver donors were so contrary to our earlier assumptions, we felt that they could no longer be swept under the rug. To us, it was simply a report of the results. Unfortunately, a whole new mind set had been infused into the field by then, and most found our contradictory conclusions inadmissable. Kissmeyer-Nielsen accepted our paper for publication in his new journal, Tissue An-

Last available clinical rank vs. matching grade for transplants between 1 January 1964 and 1 June 1970

		Clinical rank							Rank sum tests for correlation of matching (clinical comparison)
		A	B	C	D	F[a]	N[b]	total	
Sibs	A	59	15	3	3	5	5	90	A *vs.* BCDF P=0.008
	B	12	6	1	0	9	2	30	AB *vs.* CDF P=0.02
Match grade	C	46	18	4	0	18	11	97	ABC *vs.* DF P=0.07
	D	23	11	5	3	6	6	54	
		140	50	13	6	38	24	271	
Parents	A	11	10	2	0	5	2	30	A *vs.* BCDF P=0.68
	B	17	13	5	0	7	0	42	AB *vs.* CDF P=0.07
Match grade	C	55	36	14	6	24	9	144	ABC *vs.* DF P=0.05
	D	40	17	9	3	23	11	103	
		123	76	30	9	59	22	319	
Cadaver and unrelated	A	1	0	2	0	2	3	8	A *vs.* BCDF P=0.81
	B	8	1	2	0	9	4	24	AB *vs.* CDF P=0.94
Match grade	C	13	10	6	3	23	11	66	ABC *vs.* DF P=0.85
	D	40	19	11	2	41	36	149	
		62	30	21	5	75	54	247	

[a] Failure classified as from immunological causes.
[b] Failure classified as from non-immunological causes.

Figure 6. Match grade of A = no mismatched group, B = no mismatched group, but mismatched antisera, C = one mismatch, D = 2 or more mismatch. Note the excellent correlation with sibling donor transplants, but none with cadaver donor grafts. The main problem is seen in the badly mismatched transplants with good function. 1971 (40)

tigens, though the paper was published along with his skeptical editorial warning the reader about possible errors in our analysis.

I was most astonished (naively) by the financial ramifications of the report. Immediately after the Hague meeting in September, I was notified that our NIH project would be site visited, and in October, a large committee of 20 experts descended upon our laboratory, all feeling that we must be doing something wrong to have reached our conclusions. In December, I was summoned to Bethesda and told that our contract would end as of February. This was a major shock because 90% of our funds came from this one contract. In retrospect, up to that time I had been extremely fortunate in having received funds from the NIH contract group, for none of the work described to this point would have been possible without that support. Other safeguards, such as the ability to apply to the National Cancer Institute within the NIH, aided us immeasurably in the ensuing years.

The NIH Site-visit Committee made another decision. They transferred the NIH tray program that we had started for the purpose of supplying transplant centers with a uniform set of reagents from UCLA to the NIH.

In order to survive, we started to distribute trays from UCLA, but at a charge of $15 per tray. This activity continued until 1984, a period of 14 years, during which time transplants performed in the United States were, for the most part, typed using the microtest trays supplied by NIH and UCLA.

Today, about a dozen companies distribute typing reagents in the same manner using plastic trays, for it continues to be the best way to ship highly selected ready-to-use reagents. Without the microtest, this volume of typing could not be done with the available world-wide stock of antisera, for reagents are still in short supply.

In 1983 when the FDA deregulated HLA, the emergence of commercial companies forced us to cease providing trays from UCLA, a state-supported University. The core group of technicians who were producing the trays at UCLA moved outside to form the One Lambda Company. Through this source, we have continued to supply the tissue typing reagents for most of the transplants performed in the United States for an unbroken period of the past 22 years.

1971

INTERNATIONAL SYMPOSIUM ON THE ROLE OF HISTOCOMPATIBILITY TYPING IN CLINICAL ORGAN TRANSPLANTATION: PARIS, February 23-25; CHAIRMEN: JEAN DAUSSET AND FELIX RAPAPORT

This meeting was held 4 months after the Hague meeting, transparently in response to the disputed paper given at the Hague. According to the lead editorial to the volume by Dausset and Rapaport published in Transplantation Proceedings (41), "much attention has been focused recently upon the efficacy of currently available tissue typing procedures as guidelines for the selection of optimally compatible donors for clinical organ transplantation.... in response to the acute need for clarification of this situation, Transplantation Proceedings presents, in this issue a comprehensive review of the current status of histocompatibility typing in human transplantation... As a special highlight, the June issue begins with the text of a Joint International Code (Appendix I) for the analysis of the results of renal transplantation... In order to implement a collaborative effort between transplantation teams, the editors of Transplantation Proceedings organized a meeting in Paris on February 23-25 , 1971, which was attended by leading authorities representing tissue typing centers in Australia, Denmark, France, Great Britain, *The Netherlands, Norway, Sweden, Switzerland and the United States. A preliminary analysis of the results of first-set cadaver donor renal allografts performed at the participating facilities since January 1, 1969 was begun, and although the results are only of a preliminary nature at this time, they indicate that the tissue typing approach to transplantation can provide distinct advantages to the renal transplant recipient. As a consequence, this collaborative group favors the continued selection of HLA compatible donors for clinical transplantation."*

From calculations of percent mismatch in 4 serum panels, Amos showed a good correlation of outcome in 24 cadaver donor transplants ($p < 0.05$). Dausset and Hors showed a correlation in 83 unrelated transplants after consideration of cross-reactivity among 7 HLA A locus antigens and 4 HLA B locus antigens. Festenstein showed correlation in 162 cadaver donor transplants tested against 15 specificities. In 51 cadaver donor transplants, when the immunological failures were excluded, a significant ($p < 0.05$) correlation was found by Jeannet in Switzerland. Kissmeyer-Nielsen showed a good correlation ($p < 0.01$) on the basis of studies of 28 unrelated donor transplants. From studies of 128 cadaver donor transplants, Morris showed correlation on the basis of certain specificities and certain methods of calculation.

From studies of 79 cadaver donor transplants analyzed for cross-reactivity and net histocompatibility ratios, Payne came to the conclusion that more studies would be necessary. Van Rood suggested that 1) many undetected antigens were responsible for the matched transplants that failed, 2) 47 of 187 donors typed by Eurotransplant were full house, 3) bone marrow transplants showed a good correlation, 4) a high frequency of patients could receive transplants with fewer mismatches in a large pool, 5) the Eurotransplant results were 20% higher than those of the Kidney Registry (Boston), and 6) with a microabsorption technique on 28 patients, a correlation ($p < 0.037$) was obtained. Terasaki provided data showing that the microtesting methods yielded reproducible results, and that among 188 cadaver donor transplants tested for 11 specificities, a significant correlation could only be obtained for A match vs BCDF match ($p < 0.006$), but not for AB vs CDF or ABC vs DF using 11 specificities. An A match was no mismatched groups, a B match was no major group mismatch, but with more than 5% of unclassified sera mismatched, C match was with 1 specificity mismatch, D was with 2 or more, and F was a positive crossmatch.

The intent of this emergency meeting was clear: to accumulate results from all other tissue typing laboratories and thereby discredit our results. Everyone was instructed to bring raw data on typing and clinical outcome, and the data was put into a computer in Paris. The outcome of the analysis however, even excluding our results, was not favorable to the tissue typers' position. The editorial of that meeting stated that such an exercise was carried out and the preliminary results were encouraging, but no data was given. Moreover, the data has never been published! Instead, papers by different tissue typing groups were printed, all maintaining that tissue typing works. The consensus was 7 to 1 favoring the efficacy of tissue typing with 1 abstention (Payne).

The main problem which we emphasized, the paradoxical existence of mismatched patients who do well, has been confirmed in thousands of subsequent transplant patients. Moreover, none of the tissue typers today would maintain that the 10 HLA A and B locus specificities which were known in the 1968-1970 period would result in a good correlation with kidney graft outcome. Heart transplants are being performed today with the worst possible 5 and 6 HLA antigens of mismatch, dramatically emphasizing that HLA incompatibilities can be overcome by immunosuppression.

But there was some positive fall-out from the Hague. It forced us not only to learn to survive with the loss of 90% of our funding, but more importantly, to search for the reasons we failed to obtain a correlation. It was at this time that together with Dr. Mickey, we decided to create the UCLA Kidney Transplant Registry in order to follow the patients over many years. Now, 20 years later, we see that this Registry, which is the longest continuously maintained registry, has been of inestimable value in evaluating long-term survival.

In search of flaws in our approach, we postulated that some patients might be sensitized by blood transfusions. This led to the first demonstration that graft survival was lower in patients with panel reactive antibodies than in those who were unsensitized (42) (Fig. 7). Pre-sensitization for second transplants showed an even greater effect. This data has been confirmed by numerous studies and is still valid, 20 years later.

Because we suspected transfusions as one of the culprits in sensitization responsible for spoiling the HLA story, I asked Gerhard Opelz, who had just come to our laboratory, to work on the transfusion project. He had recently finished his MD degree and had some experience in a blood bank in Stockholm . Contrary to our expectations, the results were reversed, that is, blood transfusions served to increase the graft survival rate. It is to Opelz's credit that he saw this was not just a fluke, but might be a valid phenomenon (43).

In 1971, we reported that the national United States pool, started in 1969, contained 864 waiting patients in 61 different transplant centers (44). As many as 215 kidneys were exchanged among the hospitals. This computerized national communications network printed a monthly list of waiting patients. According to our 1974 report, 5,077 patients from 94 centers were listed in the national pool (45). In 1977, there were 1,600 patients in the pool from 105 centers (46). The national pool was finally discontinued in 1984 when the UNOS pool was developed.

In 1971, Dr. Setsuya Naito, from our laboratory, reported the first valid HLA-disease association. This was an increase of HLA 3 in multiple sclerosis patients (47). Later, a stronger association with DR2 was reported (48).

HLA typing for platelet transfusions and bone marrow transplants was launched in 1969 in collaboration with the National Cancer Institute (NCI). Blood sent daily in the late afternoon on a flight from Dulles Airport in Washington, D.C. to Los Angeles was picked up the following morning to be typed at UCLA. This program was to continue for a period of 17 years. During that time, over 10,000 volunteer donors at the NCI were tissue typed for the A, B and DR locus. The file of typed donors eventually contained 30,000 donors from many donor centers, including the NCI, Baltimore Cancer Center, and blood banks of Milwaukee, Albany, San Francisco, Boston, Mayo Clinic, Northwestern, Portland, Roswell Park, University of Iowa and Salt Lake City. Everyone wanted to use the pool for bone marrow transplants, but informed consent had not been obtained at that time. With this prior experience, most of these same centers cooperated later to form the National Bone Marrow Donor Registry.

The typing of blood donors introduced the problem of performing many tests at reasonable costs, which forced us to intensify our efforts to automate the microcytotoxicty test. The troublesome situation was that there were no available machines that could handle such small quantities of reagents. We were therefore forced to make our own machines. Dennis Aoki designed various machines at our laboratory. The first machine, completed in 1970, added 0.005ml of complement to 60 wells at a time at a rate of 7 seconds per

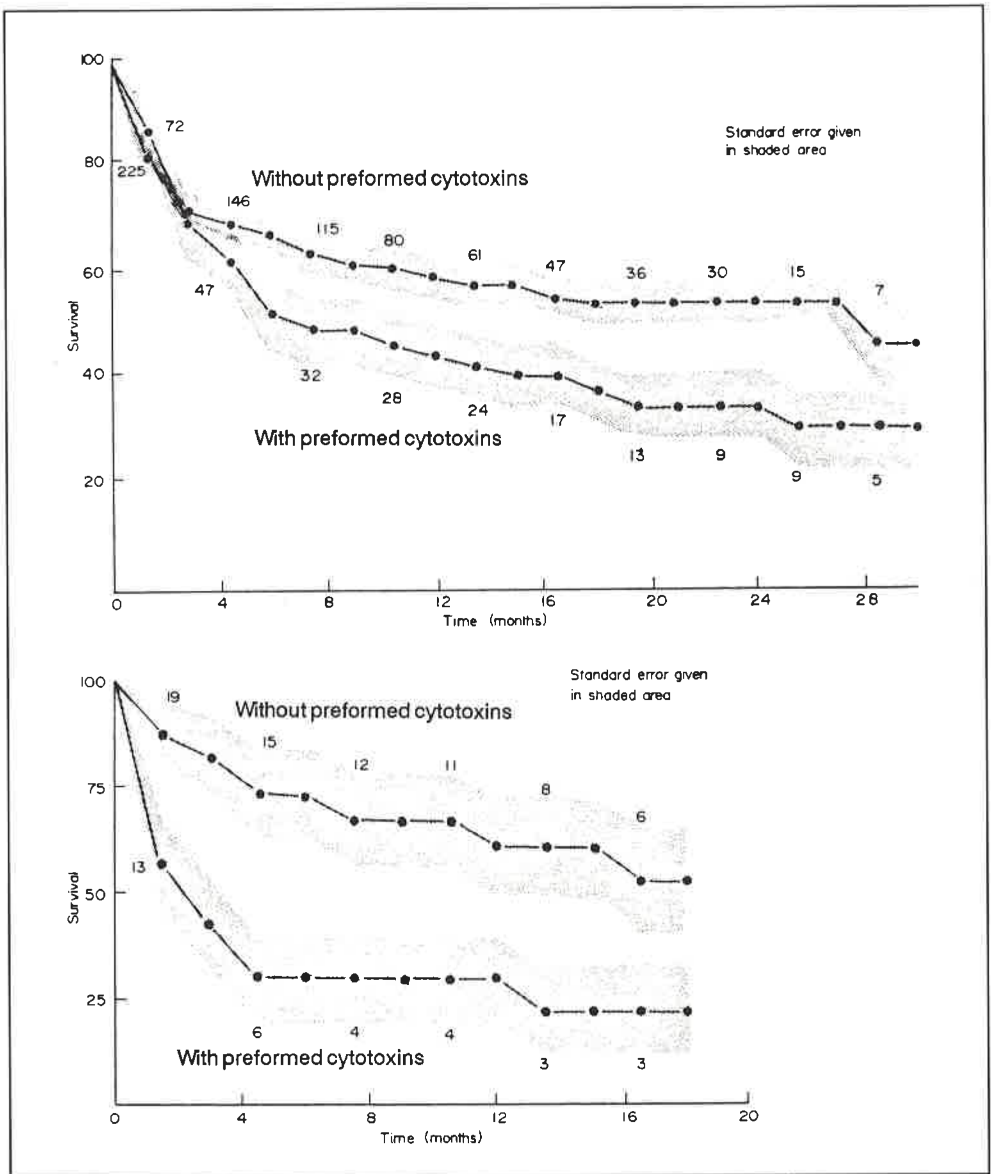


Figure 7. The effect of presensitization was first shown in this study for first and second cadaver donor transplants. 1971 (42)

tray. The next machine, made in 1971, added 0.001 ml quantities of 60 different antisera to a tray at one time, dispensing the antisera at 7 second intervals together with .005 ml of oil per tray. It required another 3 years to complete machines which added 0.00l ml of cell suspension to the tray at a rate of 6 seconds per tray. Finally, stage movers with attached microcomputers were produced in 1975. Because of the microtest and this early introduction of automation, we were able to type large numbers of patients and donors for bone marrow and kidney transplants. Patients with various diseases could also be studied for association with HLA, and thousands of sera could be screened for the much needed HLA typing reagents.

1972

INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: EVIAN, FRANCE, May 23-27, 1972; CHAIRMAN: JEAN DAUSSET

This classic workshop, organized by Dausset, examined the HLA types in 49 ethnic populations of the world by 118 highly selected antisera. Data generated by this study has remained the main reference work for frequencies of HLA in different populations for the past 20 years. Thanks to the participation of anthropologists, much could be learned about the genetic distances between the population subgroups.

Most of the Conference was devoted to the discussion of HLA typing in the populations tested. There were: indications of a third sub-locus by Svejgaard, Kissmeyer and Thorsby; the finding of lymphocytotoxic antibodies to Lewis by Jeannet and Bodmer; a description of cell-mediated lympholysis by the Ceppellini group, and the proof of independent HLA molecules on the surface of cells by Bernoco and the Ceppellini group as well as by Neauport-Sautes, Silvestre, Kourilsky and Dausset.

Dr. Donald Kuban, a former trainee in our laboratory, phoned one day to ask me if it was unusual that 4 psoriasis patients he had typed were B13. I told him he had a discovery on his hands and eventually our group and the Milwaukee group published 2 separate papers in the same issue of the New England Journal of Medicine showing that this disease had a strong association, not only with B13, but B17 as well (49, 50). This was the first disease shown to have a strong HLA association.

With the help of a contract from the NCI, we mounted a major effort to determine whether HLA associations might be found in cancer patients (Takasugi et al, 51). We tested a total of 1996 cancer patients with cancer of the lung, stomach, colon, ovary, and 10 other various sites. To this day, despite many efforts, cancer seems to be the one disease with no real HLA associations.

1973

In 1971 we moved into a new, enlarged laboratory in the UCLA Rehabilitation Building, which was built by the Rheumatology Division. To celebrate the move, we asked the rheumatologists to suggest the disease with the strongest non-sex linked genetic component, since HLA is not sex linked. Gout was proposed as the most appropriate disease. Dr. Schlosstein, a rheumatology resident, accumulated blood samples at the Wadsworth Veterans Administration Hospital. Because of the predominant male population at that hospital, and the ready availability of ankylosing spondylitis (AS) patients, Dr. Bluestone asked that the AS patients be used as controls. Since B 27 is relatively rare, occurring in 8% of the population, we were overjoyed to see so many gout patients with B27. Upon separating the controls from the gout patients, we found that it was essentially all the AS patients and not the gout patients who were B27. In fact, no HLA association could be found for gout. Because AS is a predominantly male disease, we would not have had the slightest suspicion that this disease would have any linkage to HLA. Also, the dogma that an HLA-associated disease would have to be an immunologic disease was destroyed by AS, which has no real history of being an immunologic disorder. Dr. Bluestone presented the data in November 1972 at the Western Society of Clinical Research at Carmel, California and the first publication of this finding is in an abstract from that meeting (52). We had sent the paper to Lancet, and when it was rejected there, it was resent to the New England Journal of Medicine. We were thus surprised to see that a few weeks before our paper (53) appeared, a paper showing the same association by Brewerton appeared in Lancet (54). Today, B27 is the only HLA specificity routinely used for the diagnosis of a disease.

Krain, of our laboratory, showed that pemphigus vulgaris had an HLA 10 association (55). This was further expanded many years later by M.S. Park, showing that essentially all patients with pemphigus have the DR 4 specificity (56).

In searching for a more sensitive crossmatch test for kidney transplants, Alan Ting, also from our lab, found that among the various tests examined, longer incubation of the standard microcytotoxicity test was the easiest method to gain sensitivity (57). To this day, that test is still used in many laboratories.

1974

HLA associations were examined and compared in 32 diseases (58). Our inability to store lymphocytes for more than a day was a technical difficulty for the typing laboratory. From our experience with developing a kidney storage solution, we sought to develop a better media for lymphocytes. Dr. Park finally came up with the relatively simple solution - the use of HEPES buffer in McCoys solution and room temperature storage (59). The Ph change with bicarbonate was responsible for the death of the lymphocytes. Once we realized that cells could be kept for as long as 2 weeks without losing viability, we decided to surprise our colleagues by asking them to type lymphocytes from 4 of our lab members which had been sent by ordinary air mail. We worried that many would not reply, since they might think we were checking up on them. The cells were sent to 80 labs, and everyone was so astonished that living lymphocytes could be viable after so many days, that practically everyone replied. Ninety percent of the laboratories could type the cells. The labs were in New York, Durham, Palo Alto, Denver, Cleveland, East Lansing, Boston, Columbus, Buffalo, Philadelphia, Kansas City, San Diego, Salt Lake City, Nashville, Ann Arbor, Detroit, Louisville, Madison, Miami, Milwaukee, Portland, San Antonio, Rochester, Chicago, St. Louis, Cincinnati, Oklahoma City, Honolulu, San Francisco, Richmond, Seattle, Dallas, Rochester, Minn, Iowa City, Albuquerque, Minneapolis, Indianapolis, The Bronx, Houston, Springfield, Edmonton, Toronto, Calgary, Halifax, Ottawa, Montreal, Laval-des-Rapides, Osaka, Fukuoka, Kawasaki, Nishinomiya, Nagoya, London, Sussex, Oxford, Sheffield, Manchester, Newcastle, Bristol, Cambridge, Munich, Essen, Ulm, Bonn, Beynost, Paris, Bordeaux, Basel, Geneva, Tel Aviv, Jerusalem, Cape Town, Durbin, Edinburgh, Glasgow, New Delhi, Copenhagen, Aarhus, Amsterdam, Leiden, Brussels, Oslo, Helsinki, Stockholm, Prague, Melbourne, Massa, Firenze, Carggi, Madrid, Caracas, and Singapore.

The monthly blind cell shipment activity which was initiated in March 1974, has continued on a monthly basis even into 1990 and the 15 year experience was summarized in 1989 (60). Currently over 250 labs participate and cultured B lymphocytes and antibodies are also sent in these blind studies. DNA assignment of specificities has been started so that the serological assignments can be checked against those done by PCR and oligonucleotide typing. We believe that much of the foundation for typing uniformity, which kidney shipments between centers depended upon, was laid by the 162 cell exchanges that have been sent continuously for the past 16 years.

1975

SIXTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: June 29 - July 5, 1975; CHAIRMAN: FLEMMING KISSMEYER-NIELSEN

The number of participating labs by now had increased to 74, and 178 sera were exchanged. A major component of cellular reactions was added, with Thorsby *as Chairman, and many papers dealing with the HLA-D determinants were presented.*

This was the first meeting at which we talked about a council, and one can see from the workshop volume that a list of the councillors appears for the first time. The past presidents were: B. Amos, J.J. van Rood, R. Ceppellini, P. Terasaki, and the councillors were: F.H. Allen, J.R. Batchelor, W.F. Bodmer, C.P. Engelfriet, P.J. Morris, R.D. Owen, R. Payne, Z. Trnka, and R.L. Walford.

Papers demonstrating the third locus were presented by Nielsen, Sandberg, Mayr, van Leeuwen, Ryder, Svejgaard, Layrisse, Payne, Radvany, and Grumet. Altogether, 51 HLA specificities were now identified from the Workshop results. A workshop using the platelet complement fixation test was reported, but the test seems to have been abandoned since then.

An important new development at the Conference was the many papers on unique HLA antigens present on B lymphocytes, but not on T lymphocytes. This system later became the DR antigens. There was a sudden burst of activity, all with a 1975 publication date. Winchester, Fu, Wernet, Kunkel, Dupont and Jersild (61) described non HLA antigens on B cells. Walford also published on a new system on CLL cells (62). With a double fluorescence technique, Van Leeuwen, Winchester and van Rood showed that B cells had another set of specificities (63). Legrand and Dausset described a new system of B cells (64). At this meeting, Van Rood's group described 3 alleles at this new locus. Terasaki, Park, et al reported on 4 alleles at this locus. Legrand and Dausset described a second human B lymphocyte system. Bodmer, et al made use of lymphoblastoid lines as well as CLL cells to show the B cell antigens. Walford's group reported on cross absorption analysis of serum reactions against CLL cells. B lymphocyte antigens were detected on lymphoblastoid lines by Mann, Amos and colleagues. Solheim and Thorsby produced antibodies to Ia by immunization of volunteers with intradermal injection of white cells.

Strominger, et al described the isolation of B cell specific proteins from cultured cells. Wernet and Kunkel showed that these B cell specific molecules were the MLC stimulator. McMichael, Sasazuki, Payne, Grumet, McDevitt and Kris showed an HLA B8 associated B cell antigen (now DR3) with Grave's Disease. Van den Berg-Loonen and Lucas showed an association of LD7a (now DR2) with multiple sclerosis.

In collaboration with Gershwin at the NIH, HLA B8 was found to increase in Sjogren's Syndrome (65), and this finding was later extended to DR3 (66).

1976

Although the new B-cell groups were the topic of greatest interest, Ettenger and Ting, working in our laboratory, could find no immediate deleterious effect of a B-cell positive crossmatch (67). If DR were so important, some immediate effect should have been expected. Now, 14 years later, after many subsequent studies, this conclusion has been upheld, though some feel that a longer term consequence can arise from a B-cell positive crossmatch.

1977

SEVENTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP; OXFORD, September 4-10, 1977; CHAIRMAN: WALTER BODMER

This workshop will be remembered as the one at which the HLA-DR locus antigens were established. DRw1 to DRw7 were first detected by exchange of antisera between the laboratories. The participating laboratories increased to 150, and the number of sera exchanged increased to 360. A total of 26 new specificities were assigned new designations.

1978

INTERNATIONAL FORUM: FIFTEEN YEARS OF HLA: WHAT IS THE IMPORTANCE OF HLA COMPATIBILITY FOR CLINICAL OUTCOME OF RENAL TRANSPLANTATIONS?

Vox. Sang 34: 171-188, 1978

At this moment in time, the survey conducted by the editors of Vox Sang was wonderfully instructive on the state of the art. As might have been noticed, very little seemed to have been happening in HLA matching since 1970, 8 years before. Kissmeyer and Terasaki - Opelz were the only ones to respond to the question with data (Figs. 8, 9). Dausset mentioned HLA D compatibility and transfusions. His concluding paragraph read, "Our deep conviction is that donor selection, which at the present time represents the only practical solution, should give way in the future to another method: the induction of a specific recipient tolerance for the donor antigen, but here also a basic knowledge of the HLA gene complex will be essential."

Van Rood's summary was: "HLA A and B matching can improve mean kidney graft survival significantly especially in patients with leucocyte antibodies. It seems likely that B-cell matching will improve kidney graft prognosis further. Other factors such as pretransplant blood transfusions, are of equal or even greater importance. It must be stressed, however, that more important than all the above-mentioned HLA related and unrelated facts are top clinical and surgical care."

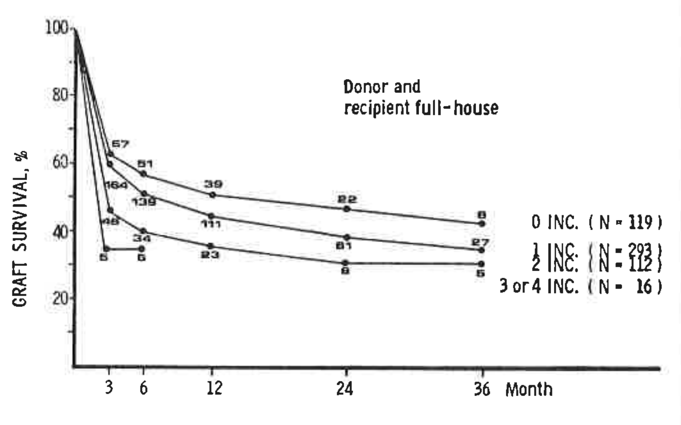


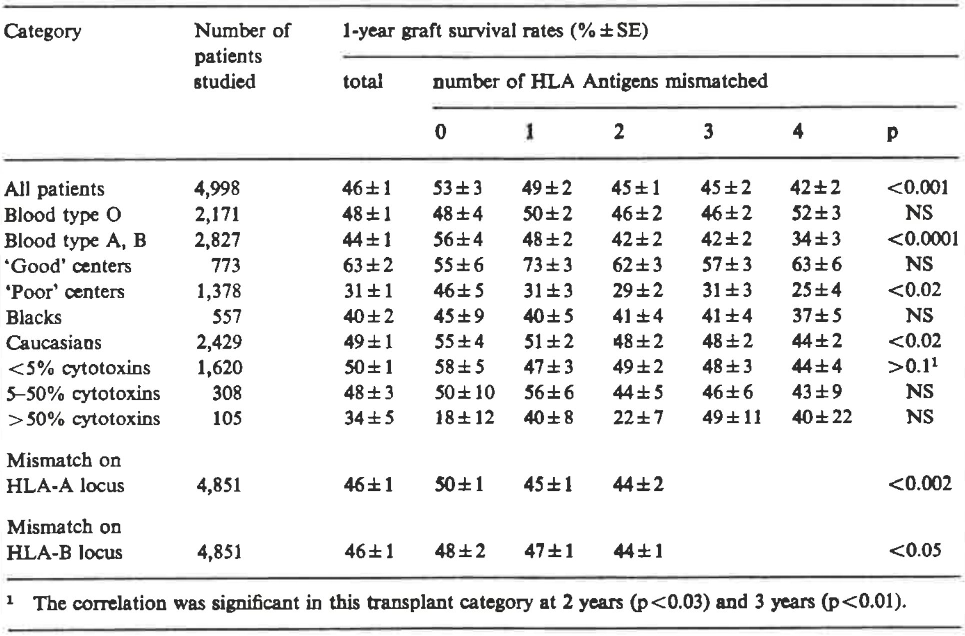
Figure 8. Survival curves of cadaver donor transplants in Scandiatransplant according to Kissmeyer-Nielsen. 1978 (Vox Sang 34:176)

It is interesting that 15 years of HLA typing for kidney transplants had brought us only to this point. The shock of the Hague was over, but hopes were now turned to the new B-cell antigens (DR).

Speiser (68), Mayr (69), Jeannet (70), Schacter and Bias (71) had published papers on the marked usefulness of HLA testing for paternity determinations. The need to keep our tissue typing laboratory going led to our evaluation of the use of HLA in paternity testing. In 1000 cases of disputed parentage, we showed that HLA was much more powerful than the conventional red cell typing (72). One of our interesting cases involved 2 dizygotic twins, shown by HLA typing to have originated from two separate fathers (73). The mother had suspected this all along, but interestingly, she had attributed the babies to the wrong fathers. Methods of computation of the probability of paternity were developed by Mickey (74) and Gjertson (75).

1979

This was the year that the Strominger group published the first amino acid sequence for HLA A2 and B7 (76). It was a major landmark, for it started the sub-



Category	Number of patients studied	1-year graft survival rates (% ± SE)						
		total	number of HLA Antigens mismatched					
			0	1	2	3	4	p
All patients	4,998	46 ± 1	53 ± 3	49 ± 2	45 ± 1	45 ± 2	42 ± 2	<0.001
Blood type O	2,171	48 ± 1	48 ± 4	50 ± 2	46 ± 2	46 ± 2	52 ± 3	NS
Blood type A, B	2,827	44 ± 1	56 ± 4	48 ± 2	42 ± 2	42 ± 2	34 ± 3	<0.0001
'Good' centers	773	63 ± 2	55 ± 6	73 ± 3	62 ± 3	57 ± 3	63 ± 6	NS
'Poor' centers	1,378	31 ± 1	46 ± 5	31 ± 3	29 ± 2	31 ± 3	25 ± 4	<0.02
Blacks	557	40 ± 2	45 ± 9	40 ± 5	41 ± 4	41 ± 4	37 ± 5	NS
Caucasians	2,429	49 ± 1	55 ± 4	51 ± 2	48 ± 2	48 ± 2	44 ± 2	<0.02
<5% cytotoxins	1,620	50 ± 1	58 ± 5	47 ± 3	49 ± 2	48 ± 3	44 ± 4	>0.1[1]
5–50% cytotoxins	308	48 ± 3	50 ± 10	56 ± 6	44 ± 5	46 ± 6	43 ± 9	NS
>50% cytotoxins	105	34 ± 5	18 ± 12	40 ± 8	22 ± 7	49 ± 11	40 ± 22	NS
Mismatch on HLA-A locus	4,851	46 ± 1	50 ± 1	45 ± 1	44 ± 2			<0.002
Mismatch on HLA-B locus	4,851	46 ± 1	48 ± 2	47 ± 1	44 ± 1			<0.05

[1] The correlation was significant in this transplant category at 2 years (p<0.03) and 3 years (p<0.01).

Figure 9. The state of HLA matching in 1978 from UCLA Kidney Transplant Registry data. Note that about a 10% difference in one year graft survival was seen in most categories of comparisons. 1978 (Vox Sang 34:184)

sequent sequencing of each of the specificities. Parham, Orr, Hurley, McDevitt, Erlich, and many others have been involved in the identification of the various specificities. As of 1990, approximately 13 A locus, 24 B locus and 14 DR locus specificities have been sequenced. This means that the exact amino acid composition of each of the HLA types detected by serology will be known within a few years.

1980

EIGHTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: LOS ANGELES, February 4 -10, 1980; CHAIRMAN: PAUL TERASAKI

This was the workshop in which the HLA - DR locus was more clearly defined, thanks to improvements in the techniques of B-cell isolation. New MT and MB specificities, that were the seeds for the new DQ locus to be identified in subsequent workshops, were also introduced. One important accomplishment resulting from the testing of 720 antisera on 37,000 cells in this Workshop, was the collection of frequencies and characteristics of all 78 of the HLA specificities. Fourteen new specificities were given WHO designations. The 1980 tables continue to be used as a reference volume, even in 1990. Much of the credit for these analyses belongs to Ray Mickey, Domenico Bernoco (ABC), and Min Sik Park (DR). Max Bauer, a guest investigator from Germany, and John Danilovs, compiled the main haplotype tables.

From our viewpoint, the central feature of the 1980 workshop was that this was the first time the Histocompatibility Workshop dealt with the problem of histocompatibility or transplantation. Until then, we had been preoccupied with the task of defining the antigens, and not with the possible relevance of these antigens to histocompatibility. A special set of antisera were set aside for transplant studies, and as part of this Workshop, 1,113 kidney transplant patients throughout the world were typed on this common panel.

According to Opelz, the HLA A and B locus mismatching did not show a correlation with 6-month survival, either in the North American or European data. However, HLA DR did show a correlation in both data sets. Moreover, DR matching affected the kidney graft survival in non-transfused patients but had no effect in the transfused patients.

Association of disease with HLA was studied in insulin dependent diabetes, chronic active hepatitis, rheumatoid arthritis, membranous glomerulonephritis, myasthenia gravis, coeliac disease, psoriasis, multiple sclerosis, hemochromatosis, and 21 hydroxylase deficiency. All these diseases had a relative risk ranging from 2 to 17. That is, if one had a given HLA type, one would be 2 to 17 times more likely to develop the disease than someone who did not have the specificity. Compared to ankylosing spondylitis, which had a relative risk of about 90, these diseases can be said to be weakly associated.

It was at this meeting that the list of international councillors was expanded to include: Drs. E. Albert, W. Mayr, M. Aizawa, F. Bach, H. Bashir, J. Bodmer, C.B.Carpenter, L. Degos, B, Dupont, H. Festenstein, P. Ivanyi, Z. Layrisse, A. Piazza, P. Rubinstein, A. Svegaard, E. Thorsby, R. Tosi, K. Tsuji and E. Yunis.

1982

INTERNATIONAL TRANSPLANTATION SOCIETY

Having been accorded the honor of being the Transplantation Society's elected President, I noted in the Presidential Address that about 40% of the kidneys were already being shared among transplant centers in the United States. With restrictions produced by ABO matching, sensitization, and the need for better HLA matching, international kidney sharing was suggested (77).

Dr. James Cicciarelli, in our laboratory, perfected improvements in the isolation of B lymphocytes for typing cadaver donors and dialysis patients using monoclonal antibodies coupled to ox red cells (78). The earlier nylon wool adherence of B cell method of Danilovs (79) was excellent for blood from normal persons, but inadequate for dialysis patients and cadaver donors.

1984

NINTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: MUNICH, May 6-11, VIENNA, May 13-15; CHAIRMEN: EKKEHARD ALBERT AND WOLFGANG MAYR

Further refinements were accomplished in the definition of HLA specificities, particularly in the DR region. DQ 1 to 3 and DP 1-6 were identified. In the Workshop study of 2973 kidney transplants typed with the same sera, Opelz noted a strong correlation with outcome. ABDR mismatching produced a 22% difference between the best and worst match at 6 months. The difference was also marked, even with BDR mismatching. These data contrasted to that obtained at the 1980 workshop, 4 years earlier. Improvement in DR typing, both in reagent quality and technical proficiency, was suggested as a significant difference.

Many papers were presented on RFLP or restriction length polymorphisms as a new way to study the HLA system. This interest carried over into the next Workshop.

1985

Ehrlich and his group presented a significant new development, the introduction of the polymerase chain reaction to the study of HLA (80). This technology, which already had enormous applications, forms the basis of intensive investigations in the area of HLA today. Some think that one day (not yet as of 1990), this test will replace the microcytotoxicity test for the determination of HLA antigens.

By 1985, over 4,000 publications had appeared on the association of HLA with disease. Moreover, over 500 different diseases had been studied. After 4 years of intensive work, Dr. Jawahar Tiwari completed the compilation of HLA associations with disease (81). Although there was a great deal of interest in this study, it turned out that at the end of some 20 years of work, the main disease with an association was still AS and its associated Reiter's disease. Pemphigus with DR4 (56) and narcolepsy with DR2 (82) were the only other diseases in which practically all the patients were of the same type. Weaker associations were found with rheumatoid arthritis, anterior uveitis, juvenile diabetes mellitus, graves disease, coeliac disease, psoriasis vulgaris, dermatitis herpetiformis, Behcet's disease, idiopathic hemochromatosis, Sjogren's syndrome, systemic lupus erythematosus, Goodpasture's Syndrome, multiple sclerosis and myasthenia gravis.

The so called 'public' determinants of HLA which include the 'private' determinants were identified in the sera of 50,000 pregnancy antisera (83). Drs. Wakisaka, Park and Konoeda postulated that these determinants were the epitopes against which the antibodies had been made. In other words, most of the antibodies produced by immunization by pregnancies, (and probably by transplantation) were directed at different epitopes on the HLA molecule. They were not mixtures or crossreactive antibodies, as was commonly thought. The frequency of occurrence and postulated immunogenicity of the epitopes were given. Dr. Park accumulated evidence that this hypothesis was correct during 1988-90, when sufficient numbers of amino acid sequences became available to match the public antibodies with sequences (84).

1986

We initiated an annual summary of the influence of HLA and other factors in Clinical Transplantation (85). This series reviewed the factors important to transplantation in 5 yearly volumes from 1985 to 1989. Data from the Registry as well as data supplied by excellent large single centers were published concurrently each year. Although the effect of HLA matching could not be seen by many of the large centers, in the pooled material from 1985 through to 1989, the effect was very clear. Thus, from sometime around 1984, there has been a good association of HLA matching with transplantation outcome. A recent figure of the association is shown in Figure 10. The difference in the various mismatch categories increases with time. The long-term half-life is better with the well-matched transplants. Festenstein had for many years noted that HLA AB typing had a long-term effect.

We are often asked why at one time we stated that tissue typing didn't work and later, changed to say it did. Our statement in 1970 for 11 HLA A and B locus specificities still holds. Now with the testing for over 60 specificities, including the DR locus, the statement can be modified (Cicciarelli et al, 86; Cecka 90). Undoubtedly with further refinements in HLA typing and more widespread typing standardization, an even higher degree of correlation will be obtained.

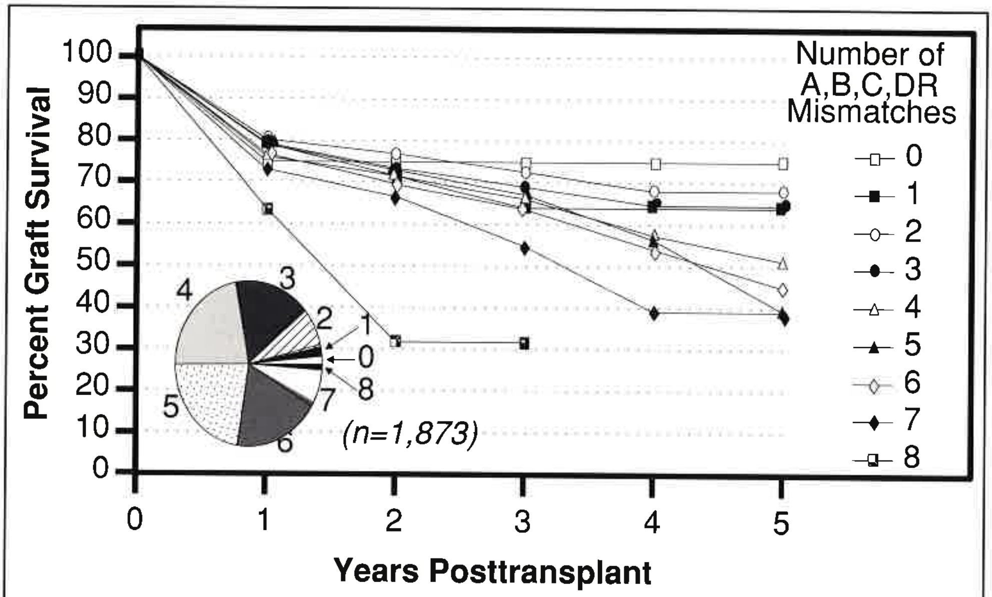


Figure 10. When the HLA C locus was considered in addition to the A, B, and DR loci, a larger separation was obtained (Gjertson, 1989). Note the lower rate of long-term graft loss in the better matched transplants. (Clinical Transplants, 1989)

The need for international HLA typed bone marrow donors was acutely felt during the Chernobyl nuclear accident. A few days after the accident, I was asked to type the victims and their family members, looking for HLA identical siblings (88). Since they did not allow any technicians into the Soviet Union during the crisis, my early experience when we were developing the test came in handy. Fortunately too, Dr. Zotikov and Dr. Zarletzkaya, both from Moscow, were former trainees in our lab who helped me type the patients. They were both competent, but were happy to receive the reagents and equipment I brought along. Aside from emphasizing the need of an international unrelated bone marrow donor panel (which was not sufficiently developed at that time to yield any matches), the incident could have contributed to easing tensions between the two superpowers.

1987

TENTH INTERNATIONAL HISTOCOMPATIBILITY WORKSHOP: NEW YORK, November 1987; CHAIRMAN: BO DUPONT

Most of the work concentrated on RFLP analysis. Extensive tables were published on the restriction enzymes and the fragments produced. Some changes in the nomenclature system were initiated at the WHO meeting to accommodate new knowledge on the polymorphisms of different chains in the DQ and DP loci. The Conference and Workshop, attended by 1,400 participants, produced 1,600 pages of new data...too extensive to be reviewed here. Some idea of how far we have come in HLA is given by the table of HLA specificities taken from a recent WHO nomenclature agreement based largely on the results of this International Workshop (Fig. 11).

Figure 11. Nomenclature of factors of the HLA system, 1989. The allele names given in the first column are based on amino acid sequences.

HLA alleles	HLA specificity	HLA alleles	HLA specificity	HLA alleles	HLA-DR specificity	HLA alleles	HLA-DQ,DP specificity
A*0101	A1	B*4101	Bw41	DRB1*0101	DR1	DQA1*0101	–
A*0201	A2	B*4201	Bw42	DRB1*0102	DR1	DQA1*0102	–
A*0202	A2	B*4401	B44(12)	DRB1*0103	DR"BR"	DQA1*0103	–
A*0203	A2	B*4402	B44(12)	DRB1*1501	DRw15(2)	DQA1*0201	–
A*0204	A2	B*4601	Bw46	DRB1*1502	DRw15(2)	DQA1*0301	–
A*0205	A2	B*4701	Bw47	DRB1*1601	DRw16(2)	DQA1*0401	–
A*0206	A2	B*4901	B49(21)	DRB1*1602	DRw16(2)	DQA1*0501	–
A*0207	A2	B*5101	B51(5)	DRB1*0301	DRw17(3)	DQA1*0601	–
A*0208	A2	B*5201	Bw52(5)	DRB1*0302	DRw18(3)		
A*0209	A2	B*5701	Bw57(17)	DRB1*0401	DR4	DQB1*0501	DQw5(w1)
A*0210	A2	B*5801	Bw58(17)	DRB1*0402	DR4	DQB1*0502	DQw5(w1)
A*0301	A3			DRB1*0403	DR4	DQB1*0503	DQw5(w1)
A*0302	A3	Cw*0101	Cw1	DRB1*0404	DR4	DQB1*0601	DQw6(w1)
A*1101	A11	Cw*0201	Cw2	DRB1*0405	DR4	DQB1*0602	DQw6(w1)
A*2401	A24(9)	Cw*0202	Cw3	DRB1*0406	DR4	DQB1*0603	DQw6(w1)
A*2501	A25(10)	Cw*0301	Cw4	DRB1*0407	DR4	DQB1*0604	DQw6(w1)
A*2601	A26(10)	Cw*0501	Cw5	DRB1*0408	DR4	DQB1*0201	DQw2
A*2901	A29(w19)	Cw*0601	Cw6	DRB1*1101	DRw11(5)	DQB1*0301	DQw7(w3)
A*3001	A30(w19)	Cw*0701	Cw7	DRB1*1102	DRw11(5)	DQB1*0302	DQw8(w3)
A*3101	A31(w19)	Cw*1101	Cw11	DRB1*1103	DRw11(5)	DQB1*0303	DQw9(w3)
A*3201	A32(w19)	Cw*1201	–	DRB1*1104	DRw11(5)	DQB1*0401	DQw4
A*3301	Aw33(w19)	Cw*1301	–	DRB1*1201	DRw12(5)	DQB1*0402	DQw4
A*6801	Aw68(28)	Cw*1401	–	DRB1*1301	DRw13(w6)		
A*6802	Aw68(28)			DRB1*1302	DRw13(w6)	DPA1*0101	–
A*6901	Aw69(28)			DRB1*1303	DRw13(w6)	DPA1*0102	–
				DRB1*1401	DRw14(w6)	DPA1*0103	–
B*0701	B7			DRB1*1402	DRw14(w6)	DPA1*0201	–
B*0702	B7			DRB1*0701	DR7		
B*0801	B8			DRB1*0702	DR7	DPB1*0101	DPw1
B*1301	B13			DRB1*0801	DRw8	DPB1*0201	DPw2
B*1302	B13			DRB1*0802	DRw8	DPB1*0202	DPw2
B*1401	B14			DRB1*0803	DRw8	DPB1*0301	DPw3
B*1402	Bw65(14)			DRB1*0901	DR9	DPB1*0401	DPw4
B*1501	Bw62(15)			DRB1*1001	DRw10	DPB1*0402	DPw4
B*1801	B18			DRB3*0101	DRw52a	DPB1*0501	DPw5
B*2701	B27			DRB3*0201	DRw52b	DPB1*0601	DPw6
B*2702	B27			DRB3*0202	DRw52b	DPB1*0801	–
B*2703	B27			DRB3*0301	DRw52c	DPB1*0901	DP"Cp63"
B*2704	B27			DRB4*0101	DRw53	DPB1*1001	–
B*2705	B27			DRB5*0101	DRw15(2)	DPB1*1101	–
B*2706	B27			DRB5*0102	DRw15(2)	DPB1*1301	–
B*3501	B35			DRB5*0201	DRw16(2)	DPB1*1401	–
B*3701	B37			DRB5*0202	DRw16(2)	DPB1*1501	–
B*3801	B38(16)					DPB1*1601	–
B*3901	B39(16)					DPB1*1701	–
B*4001	Bw60(40)					DPB1*1801	–
B*4002	B40					DPB1*1901	–

Thirty years after the first description of the Mac HLA 2 antigen, the molecular structure was unravelled by the already classical work of Bjorkman, Strominger, Wiley, et al on the molecular structure of HLA (89).

In the United States, as a result of congressional legislation, all the transplant centers have registered their prospective recipients in a single national computer and agreed to collaborate with each other in organ sharing. This organization, the United Network for Organ Sharing (UNOS), put into effect the mandatory sharing of all kidneys that were 6-antigen matched to any recipient in the country.

A national UNOS Registry was created with sub-registries of kidney, heart, liver and pancreas. The UNOS Kidney Registry was established in our laboratory by Dr. Mickey, and is currently managed by Dr. Michael Cecka, who has completed the first report from the Registry (90).

1988

At the Transplant meetings in Sydney, we presented data which clearly showed that the half-life of well-matched patients was superior to those who were less well matched (90). Thus, the major effect of HLA matching appears to take place even 1 year after the transplant.

Dr. Yuichi Iwaki called the adequacy of the crossmatch test into question by the continued high incidence of primary kidney non-function of 8% for first grafts, 14% for second and 30% for third grafts (91). When examined further by flow cytometry, (for regrafted patients), Dr. Daniel Cook found flow cytometry to be effective in reducing the primary non-function rate (92).

1989

To 1990, over 500 six-antigen matched kidney transplants were prospectively shipped through UNOS and implanted on the basis of tissue typing alone. Early results showed that the survival of these kidneys was 90% at one year (93). More recent data indicates that the 1 year survival may be a few percentage points less, but the 2-year graft survival is 83%, and the long-term half-life is 13 years. Compared to the 70% 2 year survival in controls and the 7-8 year half-life noted for the past 20 years, this represents a significant improvement. Further implementation of well-matched transplants still needs to be accomplished.

1990

We were able to add a fourth disease to the 3 with nearly complete association with HLA (ankylosing spondylitis, pemphigus, and narcolepsy); all 29 patients with primary sclerosing cholangitis were shown to have DRW 52 (94).

On the basis of the complete sequencing of the amino acids of HLA specificities, it is now possible to think of HLA matching at the level of the amino acids. Mismatching for 1-6 "antigens" of the A, B and DR locus is based on the assumption that these antigens were true alleles at the 3 loci. Now, from the amino acid sequences, we know that they are only pseudo-alleles. The true alleles and true "loci" of mismatch are the alternative amino acids present at each residue. Thus, we are usually dealing with 2 to 7 alleles at least 159 'loci', or variable amino acid sites. In the A locus, there are 55 known variable sites, 50 in the B locus and 54 in the DR locus. Computer programs have been devised to covert the conventional typing to amino acid sequences, and to match donors and recipients for these 'epitopes'. Although the true epitopes are not yet known, from the basic amino acid mismatch, and from empirical transplant results, the important epitopes for transplantation are being identified (95).

It should be noted that computers have been critical to the development of this field of work. Van Rood introduced the use of computers without which identification of the original specificities would have been impossible. Today, we are faced with the task of analyzing the antiserum reactions to each of the variable amino acids, which currently number 159. With respect to the immunogenicity of these epitopes for transplants, it is now necessary to evaluate the matching of at least 159 tissue types per patient instead of 6 HLA A, B, DR types per person.

CONCLUSIONS

Initially the most awesome task was to reveal how the antiserum reactions fit genetically into an understandable system. The culmination of the concept of a single locus and then subdivisions of it was the important first step. Although a single H-2 locus had long been established in the mouse, knowledge of serology from inbred mouse strains at that time did not help at all in untangling the complex situation in humans. The sera had to first be grouped into specificities, then assigned to the loci.

The most unique aspect of the history of HLA, I believe, is the depth and extended duration of international collaboration. A written record of this 25 year cooperation during which 10 international workshops were held is available. Perhaps even more remarkable is the fact that this core group of investigators was held together WITHOUT a formal society. In fact, it could be argued that it was only because we did NOT form a society that progress was so rapid and unimpeded.

Nevertheless, if we strip away all sentiment, there is a possibility that the high level of collaboration between the laboratories existed because it was necessary. The endless polymorphism of HLA forced us to cooperate. No single investigator could afford to go it alone. Those who tried soon found that they were left behind. Either you participated in the International Workshops, or you were not in the field. At every workshop, it was clear that the antisera gathered by others contributed to the whole picture, and any individual lab had only one part of the puzzle. The key to HLA was the antisera, and there seemed to be a limitless number, composed of all manner of mixtures of antibodies. It was these antisera which defined the HLA types as we know them today.

We had gathered together to exchange antisera and information on the similarity and differences between antisera. This was the glue that kept us together. The fervor at the workshops was in direct proportion to the importance of the antisera. One could say that this activity started at the second workshop in 1965 and continued unabated for 15 years, reaching a peak by the 8th or 9th workshop in 1980 and 1984. Sera of each workshop set the standards until the next workshop. As seen by the workshop contributor lists, many more investigators were involved than those listed as the 10 authors of this book.

If we were to ask what is HLA 1, the definition was, it is the antigen defined by anti HLA 1 antisera. And what are anti HLA 1 antisera? They are the sera which react with the HLA 1 cells. Thus, in our field, we were engaged in setting up working definitions acceptable to all. Now were are able, gradually, to provide an objective definition of the antigens based on the amino acid sequence which comprise each so-called specificity. But even so, some mechanism of naming is needed for the new sequences being uncovered daily, as shown in Fig. 11. The WHO sponsored Nomenclature Committee, led to a large extent over the past 20 years by Sir Walter Bodmer, has been a critical infrastructure for the HLA work. The other underpinning of all HLA work was the microtest, without which exchange and standardization could not have occurred.

THE FUTURE

The central question of the exact biologic role of HLA, and the reasons for such a high degree of polymorphism in the system still remains. Is there any chance that they exist only because the differences do not matter, as argued by Klein (96). With hemoglobin, one amino acid substitution is fatal or near fatal to the workings of the molecule. Is it possible that with HLA, the mutations have accumulated merely because they didn't have selection value? Most current theories would hold that, on the contrary, polymorphisms at this one locus are of cosmic importance to the very existence of the species. HLA is generally thought to function as presentation molecules vital to the presentation of peptides to the T-cell receptors, as first suggested by the work of Zinkernagel and Daugherty (97). An even more basic role as cell adhesion molecules, with a history extending back to the slime molds, has been postulated by Ohno (98). Similarly, the evolutionary origins of immunity traced to the relation between cell adhesion molecules and immunoglobulins has been advocated by Edleman (99), and reviewed recently by Hunkapiller and Hood (100). Even more unexpected developments on the role of the new HLA E, F and G loci (101) can be foreseen.

At a more practical level with respect to transplants, we are still as far away as we were 20 years ago in establishing the relevance of HLA in transplants. Over 2000 heart transplants and over 3,000 liver transplants are performed each year with no attention paid to the HLA mismatch between donor and recipient. Probably more than half of the 15,000 kidney transplants performed are also done without attention to matching. Only in bone marrow transplants is HLA matching taken seriously. The task ahead remains similar to the situation in 1970; how to identify those incompatibilities which can and cannot be circumvented with immunosuppression.

The need for collaboration continues undiminished. Experience with matching or mismatching for the extremely polymorphic HLA system cannot be gained without extensive data pooling from each of the transplant centers. Shipping of organs between centers will depend on the proficiency of the typing laboratories throughout the world in identifying the standardized specificities with the same nomenclature.

Although it is assumed that most of the HLA specificities are already known, the vast numbers of pregnancy and transplant sera whose specificities are not clearly identified with respect to the epitopes they react with, indicates that there will be a continuing need to exchange sera among laboratories to define and name new specificities.

The microcytotoxicity test has outlasted all other tests for 26 years as the method of choice for determination of the HLA specificities. It has withstood several challenges, such as complement fixation tests in 1970-75 and Elisa tests in 1984-86. G.B. Ferrara bet with me a year and a half ago that DNA technology would completely replace the microtest in 2 years. Since zero percent has been replaced to date, how many more years will it take?

Major new advances are being made today by a fresh generation, and the REAL history of HLA is still being written. My tale here has been much like the memory of my 95 year old father.... sharply detailed about days long past, but weak on recent events. This particular book on the History of HLA however, was meant to be just that... a story of how the field evolved in the olden days.

UCLA Tissue Typing Laboratory

Academic Staff	Start	Total	Academic Staff	Start	Total
Donna Vredevoe, PhD	1964	2	Kokichi Kubota, MD	1980	1
Kamal Mittal, PhD	1968	5	Ken Foon, MD	1980	2
DP Singal, PhD	1968	1	Aida Barbetti, MD	1980	4*
Ramon Patel, MD	1968	1	David Chia, PhD	1980	10*
Maria Shugarman, MD	1968	2	Steven Hardy, MD	1981	9*
Geoffrey Collins, MD	1968	2	Bing Shi, PhD	1981	1
Hans Uhse, MD	1968	1	Jessica Jephthah, MD	1981	1
Ekkehard Albert, MD	1969	2	Safwat Abdel-Baki, MD	1981	1
Federico Moscardi, PhD	1969	1	Xiu-ming Wang, MD	1981	2*
Mitsuo Takasugi, PhD	1969	21*	Xing-Pei Xu, MD	1981	1
Tsunehiko Hasegawa, MD	1970	3	Hiroyuki Togashi, PhD	1981	3
Miguel Kreisler, MD	1969	1	Pawel Kaszubowski, MD	1981	3
Setsuya Naito, MD	1969	1	Jane Galton, PhD	1981	4
Dharmendra Sengar, PhD	1969	3	J. Michael Cecka, PhD	1982	8*
Vilma Mottironi, MD	1969	1	Masaki Hirota, MD	1982	2
Tetsuya Miyajima, MD	1969	3	Kiyoyasu Fukushima, MD	1982	2
Takashi Mori, MD	1970	1	Hideo Mugishima, MD	1982	2
Nobuo Tanaka, MD	1970	1	Akihiro Sueyoshi, MD	1982	2
Greg Wilkinson, MD	1970	1	Gary Kukes, MD	1982	2
Gerhard Opelz, MD	1971	11	Takashi Iguro, MD	1982	3
Alan Ting, PhD	1971	5	Nao Suyama, MD	1982	2
Gary Rachelfsky, MD	1971	1	Akemi Wakisaka, MD	1983	2
Ronald Billing, PhD	1972	11	Darlene Katz, PhD	1983	1
Peter McConnachie, PhD	1972	1	Masaaki Kawahara, MD	1983	1
Brahma Sharma, PhD	1972	2	Po Chang Lee, MD	1984	1
Rolf Ackerman, MD	1973	1	Joseph Oei, MD	1983	2
Cristal Ackerman, MD	1973	1	Nung-su Yun, MD	1983	1
M Kiuchi, MD	1973	2	Sarina Cats, MD	1983	1
A. Zebrowski, PhD	1973	1	Kiyoshi Kasai, MD	1983	1
Min Sik Park, DVM	1974	16*	Kokichi Kawada, MD	1983	2
David Gjertson, PhD	1975	15*	Philippa Kennealy, MD	1984	1
Robert Ettenger, MD	1975	1	Toshinao Takenouchi, MD	1984	3
S. Ian Drew, MD	1975	3	Howard Takiff, MD	1984	2
Etienne Dupont, MD	1975	1	Paul Chang, PhD	1984	1
Soichiro Saito, MD	1975	2	Toshinori Ito, MD	1985	1
Mutsuo Sasaki, MD	1976	2	Daniel Cook, PhD	1985	4
Gungor Ozturk, MD	1976	2	Kazuo Tokunaga, MD	1985	2
Jawahar Tiwari, PhD	1977	12	Tadashi Shibue, MD	1985	1
Yuichi Iwaki, MD	1977	11	Kazunori Kondo, MD	1985	1
Russell Hostettor, PhD	1977	1	Guixi Wang, MD	1985	4
James Cicciarelli, PhD	1977	13*	Nabil Samy, MD	1986	1
John Danilovs, PhD	1977	5	Raynald Roy, MD	1986	1
Yukio Koide, MD	1975	3	Charif Benlahrache, MD	1987	1
Domenico Bernoco, DVM	1977	4	Sue McDiarimid, MD	1987	2
Hisashi Takahashi, MD	1978	3	Steven Levine, MD	1987	1
Rafael Oriol, MD	1978	2	Hidenori Furui, MD	1987	1
Seizoh Nakata, MD	1978	2	Yumi Terasaki, MD	1988	2*
Yong Cho, PhD	1979	11*	Hoon Han, MD	1988	2
Koichiro Ohmori, MD	1979	1	Yoko Mitsuishi, PhD	1988	2*
Brian Hawkins, PhD	1979	2	Akihito Yanagiya, MD	1988	2
T. Matsumoto, MD	1979	3	Suijia Jiang, MD	1988	2*
Mariana Israeli, PhD	1979	2	Toshio Fujioka, MD	1988	1
Chun Tsan Deng, PhD	1979	11**	Guoxian Sun, MD	1989	1
Inez Alvarez, MD	1979	2	Ji-Lao Fan, PhD	1989	1
Gill Chung, DVM	1979	1	Eduardo Lim, MD	1989	1*
Tadashi Horimi, MD	1979	3	Prasad Koka, MD	1989	1*
Jacques Hors, MD	1980	1	Yukinori Sameshima, MD	1989	1*
Tsuneo Kinukawa, MD	1980	4	Yong Chan Zhou, MD	1989	1*

UCLA Tissue Typing Laboratory

Technical Staff >5 yrs	Start	Total	Technical Staff >5 yrs	Start	Total
John McClelland	1958	32*	Trisa Dangcil	1977	7
Lynn Babbitt	1967	23*	Lee Sugich	1977	13*
Brenda McCurdy Robinson	1967	9	Roswitha Heintz	1977	9
Donna Akira Kondo	1967	20	Earl Houston	1977	13**
Joyce Yuge	1967	23*	Naiyar Jahan	1977	13*
Dennis Aoki	1969	15	Gloria McClaurin	1977	9
Linda Frank Robertson	1969	15	Kathy Helstab Houston	1977	9
Roger Smith	1969	21*	Dan Teshiba	1977	7
Jay Langston	1969	21*	Steve Takemoto	1978	12*
Rita Loew	1969	17	Calvin Chun	1978	7
Susan Shirahama Yee	1969	21**	James Dillard	1979	7
Mamie Lias	1969	21**	John Daggett	1979	11*
Emi Yoshihara Koehler	1969	20*	Emiko Komesu	1979	11*
Marietta von Diepow Bernoco	1969	7	Jay Miya	1979	6
Phil Arroyo	1970	12	Tom Root	1979	5
Dennis Kondo	1970	5	Kevin Hanson	1979	8
Ken Kinoshita	1970	5	Ellen Carnahan	1979	11*
Beverly Graver	1970	20*	Cindy Yuge	1979	11*
Jennifer Tse Chia	1970	15*	Richard Teshiba	1979	5
Sandy Perdue	1971	14	Lark Kobayashi	1979	5
Nori Sasaki	1972	18**	Kathy Baum	1979	5
Shirley Louie	1972	12	Stewart Han	1979	11**
Marie Lau	1972	18*	Theresa Quinlan	1979	6
Debbie Burgos	1972	11*	Steve Smith	1979	6
Gina Devolet	1972	18*	Alan Takeda	1979	11*
Marilyn Miyakawa	1972	16*	Ron Oblea	1979	9
Molly Arce	1972	8	Francis Nozaki	1979	5
Don Arii	1973	17**	Rick Ordonez	1979	11**
Ken Kung	1973	11	Gwyn Strange	1979	5
Annie Ramseyer	1974	16*	Michele McNamee	1979	9
George Ayoub	1974	16**	Kent Kondo	1979	6
Larry Cardman	1974	16*	Sheryl Ikuta	1979	6
Judy Bond Danilovs	1974	10	Kathy Harada	1980	6
Paul Popoff	1974	12	Theresa Jones	1980	5
Barbara Schulman	1974	16*	Christine Swanson	1980	10*
Beverly Looman	1974	16*	Ed Medrano	1980	8
Ruth Bowden	1974	12	Carmen Cruz	1980	10*
Debbie Robison Volkmer	1974	10	Rudy McMillian	1980	6
Mark Hermes	1974	16**	Diane Zettle	1980	5
Jimmy Loon	1974	16**	Terry Morris	1980	9
Madeleine Opelz	1972	10	Romel Balan	1980	10*
Cliff Wong	1975	15**	Sherry Breidenthal	1980	10*
Kathy Yoshimura Hatanaka	1975	15*	Scott Corcoran	1980	8
Bill Clark	1975	15*	Peter Sheridan	1981	7
Danny Wiesel	1976	7	Janeen Soleim Ishii	1981	9*
Alphos Toyotome	1976	14*	Kathy Lucero Concannon	1981	6
Jane Marchesi Barlam	1976	8	Diane Wong	1981	9
Steve Takemura	1976	14**	Judy Okafuji Hopfield	1981	9*
Padma Upadhyayula	1976	14*	Marsha Novak	1981	9*
Julia O'Grady	1976	6	Ed Sato	1981	9*
Carol Adams	1976	14*	Sau Wa Leong	1981	9*
Cheryl Johnson	1976	14*	Richard Tonai	1981	9*
Rose Kwok	1976	14*	Athan Roumanas	1981	6
Nadim El-Awar	1976	14**	Diana Tubao	1981	7*
Nina Yokobata	1976	8	Jerry Aoki	1981	9*
Ralfe Cookson	1977	7	Lori Tanaka	1982	8*
Shirley Walton	1977	13*	Khrystina Kaszubowski	1982	8*
Mike Cohn	1977	8*	Evelyn Price	1982	8*

UCLA Tissue Typing Laboratory

Technical Staff >5 yrs	Start	Total	Technical Staff >5 yrs	Start	Total
Jason Song	1982	8*	Glenn Terashita	1983	7
Su Hui Lee	1982	8*	Kurt Yamamoto	1983	7*
Terry Hind	1982	8**	Cris Cabacungan	1985	5*
Miyuki Ozawa	1983	7*	Valerie Dario Rossi	1985	5*

* = currently at UCLA
** = UCLA + 6 yrs at One Lambda

The first table lists the academic personnel who have worked at the UCLA Tissue Typing Laboratory for a period of at least one year. Most came to spend a postdoctoral year in the laboratory, and some stayed for considerably longer periods.

The second table lists the laboratory's technical personnel who have worked for longer than 5 years. This group has been the underpinning of all that we have accomplished, as described in this chapter. Without their steady, dedicated work on the sometimes tedious but necessary chores, none of the more visible research would have been possible.

Training, supervision and continuity in our laboratory was provided over a very long period by John McClelland (32) and Joyce Yuge (23).

During the past 22 years, Los Angeles area organ transplant distribution was coordinated by Roger Smith (21), Emi Kohler (20), Barbara Schulman (16), Gina Devolet (18), and John McClelland.

The Regional Organ Procurement Agency (ROPA) based at the laboratory, distributes kidneys to 11 Los Angeles centers for approximately 500 transplants per year.

The laboratory has typed over 338,000 patients and donors. The clinical work has been done by Lynn Babbitt (23), Annie Ramseyer (16), Marilyn Miyakawa (16), Carol Adams (14), Sheryl Johnson (14), Rose Kwok (14), Lee Sugich (13), Naiyar Jahan (13), Shirley Walton (13) and others.

Beverly Graver (20) has overseen the collection and collating of data for the Kidney Transplant Registry continuously for the past 20 years, assisted by Padma Uphadhyayula (14) and Debbie Burgos (11).

Computer support was provided by Jay Langston (21), Nori Sasaki (18), Larry Cardman (16) and Bill Clark (15) Steve Takemoto (12), Dr. Yong Cho (11), Ellen Carnahan (11) and many others.

The International Cell Exchange has been run by Jimmy Loon (16) and Marie Lau (18). Various research project support has been provided by Beverly Looman (16) Jennifer Tse Chia (15) and Alphos Toyotome (14).

Accounting problems were ably handled by John Daggett (11) and Emiko Komesu (11).

Christine Swanson (10) has provided consistent and reliable secretarial help.

Paul Popoff (12) has the distinction of being the recipient of a second kidney graft (with 6 antigens of match) that is functioning perfectly.

Tissue typing trays were supplied by members of the laboratory for over 22 years: 16 years from UCLA and the past 6 years from One Lambda. Those involved in this activity were: Mamie Lias (21), Susan Shirahama (21), Don Arii (17), Mark Hermes (16), George Ayoub (16), Jimmy Loon (16), Clifford Wong (15), Steve Takemura (14), Nadim El Awar (14), Earl Houston (13), Stewart Han (12), Ricky Ordonez (12), and Alan Alweiss (10).

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79. Danilovs JA, Ayoub G, Terasaki PI. B lymphocyte isolation by thrombin-nylon wool. In: Terasaki PI, Ed., Histocompatibility Testing 1980, Los Angeles, UCLA Tissue Typing Laboratory, 287-288, 1980.

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83. Konoeda M, Terasaki PI, Wakisaka A, Park MS, Mickey MR. Public determinants of HLA indicated by pregnancy antibodies. Transplantation 41 (2):253-259, 1986.

84. Park MS, Terasaki PI, Barbetti A, Han H, Cecka JM. Significance of the HLA mmolecular structure to transplantation. In: Terasaki PI, Ed., Clinical Transplants 1988, Los Angeles, UCLA Tissue Typing Laboratory, 301-328, 1988.

85. Clinical Transplants 1985, 1986, 1987, 1988, & 1989. Terasaki PI, Ed. Los Angeles, UCLA Tissue Typing Laboratory.

86. Cicciarelli J, Terasaki PI, Mickey MR. HLA matching and cyclosporine immunosuppressant: a strong correlation. Lancet i:267, 1986.

87. Cecka JM, Corcoran S, Takemoto S, Cho Y, Mickey MR, Terasaki PI. The UNOS Scientific Renal Transplant Registry. In: Terasaki PI, Ed., Los Angeles, UCLA Tissue Typing Laboratory, 1-8, 1989.

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89. Bjorkman PJ, Saper MA, Samraoui B, Bennett WS, Strominger JL, Wiley DC. Structure of the human Class I histocompatibility antigen, HLA-A2. Nature 329:506-512, 1987.

90. Terasaki PI, Mickey MR, Iwaki Y, Cicciarelli J, Cecka M, Cook D, Yuge J. Long-term sur-

vival of kidney grafts. Transplant Proc 21:615-617, 1989.

91. Iwaki Y, Terasaki PI, Cook D, Terashita G, Lau M. The frequent occurence of primary non-functioning kidney. Transplant Proc 19 (1):764-772, 1987.

92. Cook DJ, Terasaki PI, Iwaki Y, et al. An approach to reducing early kidney transplant failure by flow cytometry crossmatching. Clin Transplant 1:153, 1987.

93. Terasaki PI, Takemoto S, Mickey MR. A report on 123 six antigen matched cadaver kidney transplants. Clin Transplantation 3:301-305, 1989.

94. Prochazka EJ, Terasaki PI, Park MS, Goldstein LI, Busuttil RW. Association of Primary sclerosing cholangitis with HLA-Drw52a. N Engl J Med 322:1842-1844, 1990.

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96. Klein, J. Origin of major histocompatibility complex polymorphism: the trans-species hypothesis. Hum Immunol 19:155-162, 1987.

97. Zinkernagel RM, Doherty PC. Restriction of in vitro T cell-mediated cytotoxicity in lymphocytic choriomeningitis virus within a syngeneic semiallogeneic system. Nature 248:701-702, 1974.

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99. Edelman GM. CAMs and Igs: Cell adhesion and the evolutionary origins of immunity. Immunol Rev 100:10-45, 1987.

100. Hunkapiller T, Hood L. Diversity of the immunoglobulin gene superfamily. Adv Immunol 44:1-63, 1989.

101. Shimizu Y, Geraghty DE, Koller BH, Orr HT, DeMars R. Transfer and expresion of three cloned human non-HLA-A,B,C class I major histocompatibility complex genes in mutant lymphoblastoid cells. Proc Natl Acad Sci 85:227-231, 1988.

CURRICULUM VITAE

Paul I. Terasaki

PERSONAL

Born:	September 10, 1929, Los Angeles, California; Married, 4 children
Address:	UCLA Tissue Typing Laboratory 950 Veteran Avenue, Los Angeles, CA 90024 TEL: (213) 825-7651, FAX: (213) 206-3216

EDUCATION

1945	Gila River Camp High School, Arizona
1946	Hyde Park High School, Chicago
1948	A.A. - University of Illinois
1950	B.A. - University of California, Los Angeles
1952	M.A. - University of California, Los Angeles
1956	Ph.D. - University of California, Los Angeles

POSITIONS

1955-56	Junior Research Zoologist, Department of Surgery, UCLA
1957-58	Postdoctoral Research Fellow, USPH Department of Zoology, University College London, with P.B. Medawar
1958-61	Assistant Research Zoologist, Department of Surgery, UCLA
1961-63	Associate Professor of Surgery-in-Residence, Department of Surgery, UCLA
1963-69	Professor of Surgery-in-Residence, Department of Surgery, UCLA
1973-	Director, Southern California Regional Organ Procurement Agency
1969-	Director, UCLA Tissue Typing Laboratory
1969-	Professor of Surgery, Department of Surgery, UCLA

MEMERSHIPS

1968-	World Health Organization: Nomenclature Committee for Leukocyte Antigens
1967-70	National Institute of Allergy and Infectious Diseases: Transplantation and Immunology Committee
1980-84	American Society of Transplant Physicians
	American Society of Transplant Surgeons (Honorary Member)
1972-78	Councillor, International Transplantation Society
1982-84	President, International Transplantation Society
1970-89	International Histocompatibility Society 1970 Secretary 1970-72 President 1978-80 Secretary 1980-84 President
1988-89	President, American Society of Histocompatibility and Immunogenetics

1987-89 United Network for Organ Sharing (UNOS) Histocompatibility Committee

1987	Director, National Kidney Transplant Registry
1987	Scientific Advisory Committee
1989	Board of Directors

JOURNAL EDITORIAL BOARDS

Transplantation
Clinical Nephrology
Immunogenetics
Transplantation Proceedings
Human Immunology
Contemporary Nephrology

AWARDS

1970	Netherlands Red Cross Medal
1971	Modern Medicine Award for Distinguished Achievement
1973	UCLA Alumni Distinguished Achievement Award
1977	American Society of Clinical Pathologists: Philip Levine Award
1988	American Society of Histocompatibility and Immunogentics: Rose Payne Award

PUBLICATIONS

Total number: 750

Citations (from the Scientist)

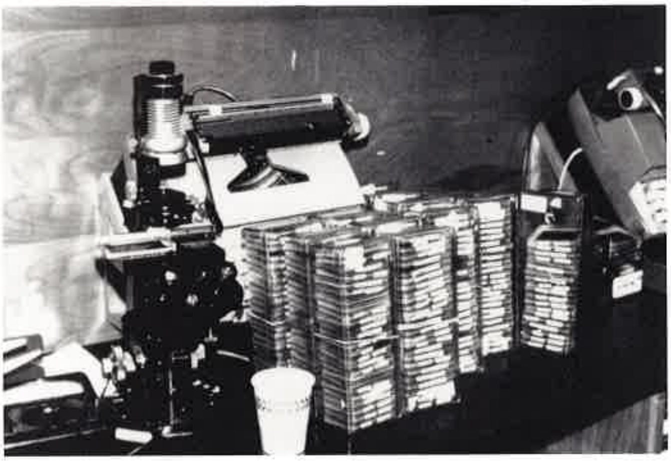
1965-1978	rank #55	7,379
1973-1984	rank #61	8,506

Thomas Starzl Paul Terasaki
Waiting for the histo train to come in,
Rehab Building UCLA

Peter Medawar 1958
pre knighthood
and Nobel Prize

In front of
Department of Zoology,
University College, London

John
McClelland
ca. 1970



Electric typewritters
attached to
microscope with
2, 4, 6, 8 keys

endless trays to
be read

1967

WHO Meeting Geneva, 1969

Z. Trnka *Ruggero Ceppellini* *Bernard Amos* *Jean Dausset* *Andre Govaerts* *Flemming Kissmeyer-Nielsen*

John Humphrey *Sir Michael Woodruff* *Donald Kayhoe* *Paul Terasaki*

"Typing" microscopes and trays to be read 1969

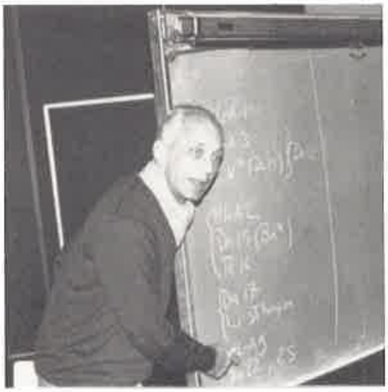
Automation engineer Dennis Aoki

Original dotting machines

Kunzo Orita Paul Terasaki Setsuya Naito

1971

1970 Linda Frank-Robertson D.P. Singal

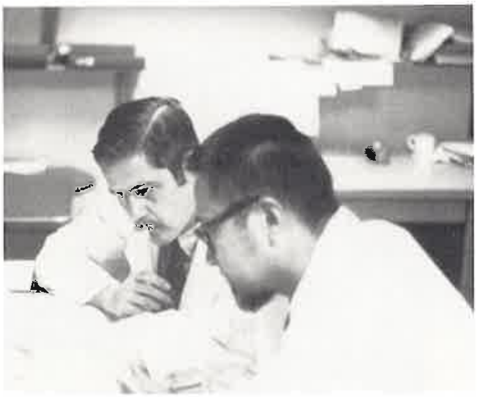


This is the way

Taller than most

Fourth Workshop, Los Angeles, 1970

Miguel Kreisler Ruggero Ceppellini Ekkehard Albert



Miguel Kreisler Paul Terasaki

Rose Payne Michel Jeannet

Michel Jeannet Ruggero Ceppellini

Rose Payne Richard Batchelor

Alan Ting dreaming it up and Peter Morris creating castles in the sky

Ruggero Ceppellini having

difficulty speaking

because of a broken arm

Fourth Workshop Los Angeles 1970

Geoffrey Collins

Fourth Workshop Los Angeles, Palm Springs 1970

Walter Bodmer

Roy Walford

Jon van Rood

Rose Payne

Wolfgang Mayr

Bernard Amos

Walter Bodmer

Paul Terasaki

Roy Walford

Jon van Rood

Paul Terasaki

Carol
Davidson
HLA logo designer

Linda Frank

1970

Paul
Terasaki

Don
Arii

Judy
Walker

ca. 1975

A. Wakisaka   Paul Terasaki   Min Sik Park
Prof. Kikuchi

Here's to

Eurotransplant

Bernard
Cohen

Flemming Kissmeyer   Erik Thorsby   Ray   Paul   Marietta
Rome, 1978   Domenico Bernoco   Mickey   Terasaki   Bernoco

Eighth Workshop

Los Angeles
1980

van
Rood
receiving
an award

Alan
Ting
in his
bachelor
days

Master
of
ceremonies
for
awards

George
Ayoub

Raphael Oriel

Emiko Terasaki
First Paternity
Case

Left to right
M. Aizawa
A. Wakisaka
Hisako Terasaki
Paul Terasaki
K. Itakura
N. Tanigaki
Yuichi Iwaki
Mrs. Iwaki

Ekkehard Albert Paul Terasaki

Ninth Workshop Munich, 1984

Hilliard Festenstein 1984

Min Sik Park

Sailing down the Rhine

Paul and Hisako Terasaki

Christmas Reindeer Michael Cecka

Arranging to transplant some kidneys ca. 1975

Paul Terasaki Thomas Starzl Yuichi Iwaki Paul Taylor

Domenico and Marietta Bernoco

John McClelland
1980 Workshop serum shipments

50 year party

Jawahar Tiwari

After 4 years of hard labor on HLA and Disease book

M.R. Mickey
as he appeared
1964-1990

Statistics team

David Gjertson

William Longmire
UCLA
Dept. of Surgery
Chairman

Madeline, Mona, Henrik and Gerhard Opelz 1982
After 11 years and 100 papers

Turtle Bay, Hawaii, 1989

Kamal Mittal

Jim Cicciarelli

G.B. Ferrara Soothsayer